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AN EXPERIMENTAL INVESTIGATION  
into  
THE HISTOLOGY of INFLAMMATION and REPAIR  
illustrated in  
THE HEALING of INCISED WOUNDS  
and  
THE EVOLUTION of an ABSCESS.

A THESIS PRESENTED for the DEGREE of M.D.  
by

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VOL. II.

Drawings and Photographs by RICHARD MUIR.

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## MICROSCOPICAL PREPARATIONS.

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BOX I. accompanying this thesis, contains the slides from which the drawings and photographs were taken (1 - 54); also, slides illustrating the various staining methods used. (55 - 117).

The remaining slides referred to in the text are deposited in the Pathological Department, under the care of Professor Greenfield, and may be had at any time from Mr Richard Muir.

BOX II. Slides 118 - 224 illustrate especially:- the formation of new blood-vessels; fibril formation; giant-cells; changes in muscle, elastic, and fat tissues.

BOX III. Slides 225 - 331 illustrate the general structure of incised wounds and abscesses at various stages.

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## P R E F A C E.

This thesis embodies the work which I have carried on during the last two years as Carnegie Scholar in the Pathological Department under the superintendence of Professor Greenfield. It is practically a continuation of my thesis "Some Problems in relation to the Healing of Wounds" which was awarded the Syme Fellowship last July. The object of that work was to present a consecutive view of the chief problems in relation to the healing of wounds, and to state briefly the various theories suggested for their solution. A short account was given of the work on which these theories were based, together with a brief description of my own preparations which I then had not had time to study. During this past year, I have completed a new series of preparations illustrating the healing of incised wounds and the evolution of an abscess, and my present paper is concerned with the morphological elements connected with these processes. By permission of the Dean, I am allowed to send in for reference the volume of drawings illustrating my former thesis, and to this I/

I have added another volume of drawings and photographs. These two volumes illustrate from my own preparations most of the important aspects of the healing process.

I hoped to have been able to send in a series of preparations illustrating the healing of intestinal wounds. As I had permission to operate only on rabbits, it was found that the small intestine is too narrow and too thin to allow of any success. Thirty operations were performed; a few of these were complete anastomoses, the others were simple longitudinal and transverse incisions. Two series of sutures were used, the one invaginating the mucous membrane, the other drawing the peritoneal surfaces together. It was found that by this means the lumen of the small intestine was so reduced that most of the animals died of obstruction. The tissues both of the intestinal and skin wounds were in every case taken through and sections cut, but, as I have no results of any value from this series, I am leaving it out of consideration in my present work.

A Grant towards the expenses of this Research was given to me from the Moray Fund.

## I. I N T R O D U C T I O N.

In spite of many investigations on the histogenesis of Inflammation, this subject still remains the battleground of pathologists. The vast amount of work done serves to show how difficult is the interpretation of the process and seems to indicate that on many points it is impossible to give established results.

As the work on which this paper is based is concerned, not with the phenomena of Inflammation and their significance, but with the morphological elements connected with the processes of Inflammation and Repair, I shall at once pass to outline the plan on which I purpose to discuss my subject.

The two main divisions are (1) the Healing of Incised Wounds and (2) the Evolution of an Abscess. Each has been divided into stages which are arbitrary but yet not quite artificial. The recognition of the phenomena of Inflammation and the treatment are the logical outcome of considerations brought forward by pathologists, and the deductions of histo-pathologists must play an increasing role in the explanation of/



of certain as yet little understood modes of treatment.

I. In tracing the healing process in wounds I have endeavoured to relate each stage to one governing idea. This one outstanding feature is not to be regarded as confined to its stage but simply to form the essential characteristic.

Every injury is followed by certain vascular changes which as a rule are included under the term Inflammation. Cohnheim in his theory of Inflammation laid special stress on these vascular changes. In the first stage the vascular phenomena which we associate with Inflammation:- the hyperaemia, the stasis, the transudation of lymph, and the emigration of the leucocytes are all very marked. This is the stage of the vascular phenomena.

Every injury, however small, has resulted in some tissue degeneration. Before any reparative phenomena can occur the irritant causing the damage and the degenerated tissue elements must be attacked. This defence of the organism in the case of a simple wound is effected chiefly by phagocytosis. Metchnikoff has showed how in all organisms, even the lowest, migratory cells flow on the damaged part and carry/

carry on their function. The second stage is that of the reign of the phagocytes.

When the two previous phenomena have to a certain extent prepared the ground for the formation of a new tissue to restore the Status quo there follows the reaction of the local tissue elements. Both connective tissue cells and endothelial cells share in this and by its means a granulation tissue is formed. The proliferated endothelium forms new vessels which assist in absorption and form a temporary scaffolding for the building up of the new tissue which will result from the proliferation of the connective tissue cells. This is the stage of the reaction of the tissue elements.

In the next period is traced the further development of this granulation tissue into layers of spindle-shaped cells. This is the stage of fibril development. The final stage is that of the condensation of these fibrous laminae, when a new tissue almost similar to the old has arisen.

The times which have been given for the different periods have been stated to be arbitrary. Many circumstances alter the sequence of the phenomena, especially those connected with the resistance of/  
of/



of the tissue to the injury and those which I have mentioned in more detail in the next chapter. In surgical pathology healing by "immediate union" is recognised and this is the probable method of healing of an uninfected small skin wound. The vascular and phagocytic phenomena are slight and the main part in the healing process is taken by the tissue cells immediately adjoining the incision. In experimented-on animals this "immediate union" is naturally rarely seen. In only a few of my wounds did actual supuration occur, but there was considerable acute inflammatory reaction in every case.

The consideration of the morphology of the elements appearing on the inflamed area would naturally raise questions as to their origin and the role assigned to them. The origin, function, and destiny of the very varied cell-forms are still undecided although more research has been expended upon this than upon any other branch of this subject. The ordinary polymorpho-nuclear leucocytes are admittedly derived from the blood-stream but the mononucleated cells -- have they also emigrated from the vessels or, to go to the other extreme, are they derived solely/

solely from fixed tissue cells or from both sources? Again, the changes in the vessels which result in new vessel-formation -- how are these brought about, and do other than endothelial cells share in their formation? Further, among the numerous cells present in the granulation tissue -- a tissue which is essentially connected with the building up of new tissue -- which cells form the new tissue? Is it only the descendants of pre-existing connective tissue cells, or do endothelial cells of blood-vessels and lymphatics or mononucleated cells from the blood share in this formation? How also is the fibril formation effected which results in the laying down of fibrous laminae? Further still to be considered are the changes occurring in the muscle, elastic, and fat tissues.

II. In studying the evolution of an abscess I have been guided by the conception of an abscess as the localisation of an acute infection. Like the inflammatory process generally it is essentially beneficial to the organism. The injection of the irritant - the *Staphylococcus pyogenes aureus* - fulfils the general law by which the same agent determines/

determines in its immediate neighbourhood destructive effects, and in a further off zone reaction and new-formation. The processes to be traced histologically can be related to what we know clinically. The bacterial toxins which called forth the original necrosis call forth the inflammatory reaction which results in healing.

The necrosis of the tissues followed by the grouping of the leucocytes around the cocci results in the formation of pus, which includes all the elements slain in the battle. This is the first stage.

The leucocytes form a barrier around the bacteria but they alone are insufficient as a defence for the organism and in the tissue around arises that reaction by which the abscess is marked off. This rampart of leucocytes and other cells marks off the pus from the zone of reaction. This is the second stage - the delimitation of the abscess.

In the reactive zone is next laid down a granulation tissue membrane which is early differentiated into two layers - an inner and an outer. In the inner occur those absorptive processes which lead to the resorption of the pus, and the vascularization/

vascularization of this membrane forms the third stage.

In the outer layer occur those processes which lead to the encapsulation of the pus mass from the surrounding tissue. This cicatrization of the membrane is the fourth stage.

These two processes occur side by side and finally lead to the complete absorption and substitution of the pus.

The histological picture helps to some extent to explain two methods of treatment which are coming more and more into surgical practice for Bier's treatment by passive hyperaemia and the "Vaccine" treatment seemed at first to rest on a frankly-admitted empirical basis.

On reaching the tissues the bacteria encounter normal defensive mechanisms, of which phagocytosis and bacteriolysis are best understood. Both take place through the united action of cells and fluids. The concentration of the leucocytes and of the serum about the bacteria serves to protect the body and to promote healing for, as they develop in the tissues, specific substances are liberated upon which depends the inflammatory reaction/

reaction. Specific antibodies, which further healing, are also locally produced.

The promotion of passive hyperaemia acts chiefly by keeping in the part the leucocytes and their products upon which so much depends and increasing the amount of anti-bacterial substances and antibodies.

In the treatment by vaccines an emulsion of the (dead) cocci, which produced the pus, is injected to aid the proliferative and reactive processes. For example in a carbuncle, the antibodies produced by the cocci are not of themselves sufficient to draw out the reactive phenomena necessary for cure. An emulsion of dead cocci is therefore injected so that by their vis a tergo action they may aid the local vis a fronte action.

Theories of Inflammation do not form part of this paper, yet the brief outline I have given of the histological picture shows that the governing ideas of the exclusive theories have each their counterpart in it. All the views contain an element of truth and are but the different aspects of some complex phenomenon observed from different points of view. Cohnheim's work laid the foundation of most of the later discoveries on the nature of inflammation and his conception comes nearest to the clinical and/

and therapeutic point of view. Phagocytosis, considered as a general process of defence for the animal organism against bacteria, is a conception which belongs to Metchnikoff. To Virchow, the founder of cellular pathology, we owe the full recognition of the living cell in all the processes of life.

Closing chapters in this division deal first with the structure of granulation tissue in detail, as it is found in an abscess membrane or on the surface of an ulcer; and lastly a chapter on the origin and significance of the so-called "round-celled infiltrations" which are found almost as small lymphoid nodules in the walls of chronic abscesses.

The closing section in this division deals in detail with the structure of granulation tissue as it is found in an abscess membrane or on the surface of an ulcer.

The closing chapter contains the conclusions to which this histological study has led me regarding some of the points discussed.



II.            PLAN OF RESEARCH AND  
                  HISTOLOGICAL TECHNIQUE.

The Plan of research was the following:-

I.        Incised wounds were made in the rabbit through the whole thickness of the anterior abdominal wall about one inch from the middle line. The animals were killed after the following periods:-

6 hours, 12, 18, 21, 24, 30, 36, 48, 60, 72, 84, 96 hours; 5, 6, 8, 10, 12, 15, 18, 20, 22, 25, 31, 40, 50, 55, 60, 70, days. Owing to the difficulty of cutting the sections, if stitches were superimposed, the whole thickness of the abdominal wall was taken in by single stitches placed close to one another to prevent hernia. It was found necessary to place the whole piece of tissue, enclosing the incision, in the fixing fluid en bloc, as the cutting of the tissue to place a portion in different fixing fluids or to obtain better penetration, disarranged the soft parts in the wound-cleft and around it. Three series of operations for each period, up to and including six days, were done and the tissues fixed/

fixed in 10% Formalin, Corrosive, and Zenker's fluid respectively. For the periods between 8 days and 70 days only one series was found necessary. The incision was divided into three parts, two of which were fixed in Zenker's fluid and one in Corrosive.

A few incisions were made in the rat and mouse for the earlier periods up to 4 days. Incised tissues were kindly given me by Professor Beattie from the dog, and by Dr. Milne from the cat and guinea-pig.

II. Five c.c. of a 24 hours' broth-culture of *Staphylococcus pyogenes aureus* was injected subcutaneously into the lateral abdominal wall of rabbits to trace the evolution of an abscess from its earliest beginnings to the complete absorption and substitution of the pus. The animals were killed after 6 hours, 12, 18, 24, 30, 36, 48, 66, 84 hours; 6 days, 8, 10, 12, 15, 17, and 25 days. Three pieces of tissue were fixed from each period, one in Corrosive and two in Zenker's fluid - one part from the centre of the tissue and one part from the periphery.

A second, shorter, series of abscesses was produced/



produced by the insertion of infected catgut into the subcutaneous and muscular tissue of the back in rabbits. The catgut was prepared by steeping in broth infected from a fresh culture of *Bacillus coli communis* and left in the incubator for 24 hours. After removal it was washed repeatedly in sterile distilled water. Sterile catgut was placed in a similar position on the opposite side of the spinal column. The animals were killed after 30 hours, 60 hours, 90 hours, 5,  $6\frac{1}{2}$ , 8, 11, 15, 18, 25, 31, and 42 days.

One or two additional abscesses were obtained in cases where the skin incisions had become septic.

It may not be out of place to mention some of the difficulties which were met with in the course of this work. In addition to the restlessness of the experimented-on animals interfering with the healing processes, there were many circumstances which altered the sequence of the phenomena. In rabbits the "skin" is composed of epidermis, a very dense corium layer closely penetrated by hairs and with few blood-vessels, and a narrow strip of muscular tissue. This is separated from the dense fascia/

fascia covering the deep muscles by a very loose layer of areolar tissue; it is in this loose layer that the separation takes place when a rabbit is "skinned". As in many rabbits the dense connective tissue of the corium had almost a cartilaginous consistence the incision produced very slight reaction, while extensive haemorrhage and leucocyte infiltration had taken place into the loose layer. It was found almost impossible to cut these sections with the microtome as the densely packed hair root sheaths left little tissue to be penetrated by the paraffin. As the most important layer for examination lay between two dense structures, the dense muscle fascia and the still denser corium it was frequently entirely broken up. A second difficulty lay in the approximation of the wound edges, due chiefly to the separation of the layers during respiration. The thin "skin" over the abdominal surface and the differing tension of its surface caused great incurving of the skin into the wound-edges. The stitches, especially in the wounds of the earlier periods, caused great difficulties in the subsequent cutting of the sections.

Many of these difficulties would have been obviated/

obviated had I been able to make the incisions on the dog, in parts where the fat tissue renders the skin wound more comparable to the conditions in the human skin, and where approximation of the wound edges might more easily have been obtained without too many stitches. On the other hand, it was found that the skin tissues of the dog are even more difficult to cut with the microtome than those of the rabbit, as these are incomparably more difficult than those of rats and guinea-pigs. By carrying the tissue through hard paraffin and embedding it in hard paraffin it was found that the dense tissues were better penetrated than when a mixture of hard and soft paraffin was used.

As simple aseptic wounds show little reaction no attempt was made at asepsis. This would in any case have been difficult in the conditions under which the operations were carried out. Many of the earlier of these were performed alone and the catgut or horsehair stitches came much in contact with the surrounding objects in my attempt to keep the animal under chloroform. The result is that many of my specimens show an excessive accumulation of leucocytes around the stitches, while knives, soon/

soon blunted by the tough skin of the rabbit, caused greater haemorrhage and excessive degenerative processes.

The further course of the healing process, the intensity and extent of the emigration of leucocytes and proliferation of cells, the time of the new formation of blood-vessels and of the transformation of the cell into fibres all varied in individual cases within wide bounds and without one being able always to discover sufficient grounds for it. Changing mechanical, chemical, and bacterial irritation took their part in these differences, even when one had taken care to carry out details in the same operation with the greatest possible equal care and technique.

A further difficulty was met with in the opening up of many of the wounds during the spasms which precede death. In consequence of this a great part of the valuable material for examination, lying between the wound-edges, fell away when the tissue was placed in the fixing fluid.

The animals were killed under Chloroform and the tissue, rapidly excised, was placed immediately in fixing fluid at body temperature as recommended/

recommended by Maximow and Schwarz. The first series of wounds was fixed in Formalin, the second in Corrosive, and the third in Zenker's fluid. The last has been by far the most satisfactory, preserving the tissue elements with little shrinkage and fixing cells in the most intensive amoiboid movement or during the different phases of mitotic division. Zenker's fluid has two drawbacks;- the sections do not stain so beautifully with any of the stains, except the Iron Haematoxylin and Unna's Polychrome Methylene Blue, as after Formalin or Corrosive; and the tissues thus fixed seem more difficult to cut with the microtome. This latter is a very serious drawback in dealing with skin tissues which are so difficult to cut however fixed.

#### HISTOLOGICAL TECHNIQUE.

The following staining methods were used:-

1. Haematin and Eosin - as a routine stain. H.E.
2. The Unna-Pappenheim Methyl-Green-Pyronin and Resorcin stain. M.G.P.
3. Heidenhain's Iron-Haematoxylin stain. I.H.

Of the large number of stains used these three gave the best results and were employed for all sections.

The other stains used were:-

4./

4. Alcoholic Eosin and Methylene Blue and Richard Muir's modification of this stain for Granules. E.M.B.
5. Benda's Stain (Saffranin und Licht Grün). B.
6. Van Gieson Stain. V.G.
7. Unna's Polychrome Methylene Blue. P.M.B.

Special methods such as Mallory's for connective-tissue fibrils, Gram's for organisms, and Weigerts' for elastic tissue were used where necessary.

Various modifications of triple stains were tried with more or less perseverance, especially Maximow's Haematoxylin, Fuchsin S., and Aurantia. A special effort was made with Schridde's modification of Altmann's stain. Schridde<sup>"9</sup> claims that by this method a differentiation of the various forms of cells within the tissues may be obtained by means of the variations in size and tint of the granules. A long series of tissues was carried through by the Altmann-Schridde method. Owing, however, to faulty technique somewhere I was unable to get the desired results. Adami<sup>1</sup>, who has seen Schridde's preparations, seems inclined to accept his conclusions.



### III. THE HEALING OF INCISED WOUNDS.

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#### (1). CELL FORMS in the SUBCUTANEOUS TISSUE of the RABBIT and RAT.

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The aim of my work has been briefly indicated in the introductory chapter - to follow step by step the healing process from the time of the injury to the restitutio ad integrum. It has already been stated how important in this relation is the question of the cells of the inflammatory exudate, and, this being so, a description of the cells found in the normal subcutaneous tissue seems justified. I studied sections from six normal rabbits to compare my results with those of Maximow<sup>75</sup>, who has given a careful description of the cell-forms of the loose connective tissue.

In a cross section of the abdominal wall of the rabbit we at once recognise the epidermis and the serosa and between these the following layers:-(1). the very dense corium penetrated by hairs and containing numerous hair follicles and glands. In the corium the cells are very isolated and of thin, flattened/

flattened form closely applied to the collagen bundles. Around the vessels a few rounder cells may be found.

- (2). Between the corium and the deep muscles is seen a layer of loose connective tissue containing near the corium the superficial muscle - the panniculus carnosus. In this loose connective tissue are more numerous vessels. The cells are not so atrophied-looking and are more numerous, especially around the vessels.
- (3). We next come to several muscle layers covered by dense fascia both towards the subcutaneous tissue and the peritoneal surface. A loose connective tissue with a few cells forms the perimysium.
- (4) Finally we come to the peritoneal endothelium separated from the muscle by dense sub-endothelial layers of connective tissue reinforced by elastic tissue with numerous lymphatics early made visible in inflammation.

Preparations illustrating cross-sections of the abdominal wall of the rabbit and rat-- stained with H.E.; I.H.; P.M.B.; M.G.P.; and E.M.B. will be found in Box II.

In the loose subcutaneous tissue are two distinctly/



distinctly defined types of cells:-

- (1). The usual connective tissue cells. These spindle-shaped elements are closely applied to the collagen bundles and have an oval nucleus. The nucleolus is only with difficulty made out in the resting cell. The structure of both nucleus and cell-body is best brought out by I.H. - the fine chromatin particles of the nucleus are connected by linin threads forming a network and around the nucleus the protoplasm has a definitely reticular structure. This portion of the protoplasm stains more darkly with P.M.B. than the nucleus. The cell processes are often indistinct. On cross-section these cells appear as round cells but can always be distinguished from the second cell-form by their clearer nucleus.
- (2) The round "wandering cell" first described in the connective tissue by v.Recklinghausen. These cells can not be distinguished from the ordinary blood lymphocytes, they have a densely-stained nucleus but a network can be made out in the nucleus of the larger forms of these cells. The Protoplasm border is very narrow and stains a pale blue with P.M.B., is pale and structureless/

structureless with I.H., and with H.E. stains faintly with the Eosine. These cells are extremely few in number in the rabbit but more abundant in the rat especially near the vessels in the loose tissue. (Plate 19). The larger forms described by Maximow where the nucleus is becoming indented are very scarce.

- (3) Maximow describes a third cell-form normally present - this is the clasmatocyte. This may be looked upon as a transition form in appearance, both in regard to nucleus and cell body, between connective tissue cells and the "round" cells. Its special characteristic is the presence of granules in the protoplasm and the cell body shows a fine-meshed network with many clear vacuoles. The nucleus is denser, the contour more defined, and the cell-body smaller than the connective tissue cell. Maximow states that in inflammation they round themselves off and add to the number of the round wandering cells. Ranvier, who first described these cells in the Omentum also stated that in inflammation they become round cells.

In addition to these cell elements distributed in the meshes of the tissue are found around/

around the vessels and hair follicles a few cells the character of which is difficult to define.

Marchand\* has given to them the name "Leucocytoid adventitial cells" and Maximow\* has divided them into (1) small a-typical connective tissue cells; and (2) clasmatocyte-like adventitial cells.

I have found no indication in the subcutaneous tissue of any perivascular lymphoid nodules such as Ribbert<sup>108</sup>\* and Beattie<sup>12</sup>\* have described in the Omentum.

The cells of the loose connective tissue are therefore:-

(1). The normal connective tissue cell.

(2). The round "wandering cell".

(3). Clasmatocytes in the tissue and Clasmatocyte-like adventitial cells around vessels. Neither of the last two groups satisfy any precise conception.

Small groups of fat cells occur in the loose tissue in the rabbit and in the rat the panniculus carnosus lies between layers of delicate areolar tissue.

In the rat we also find Ehrlich's Mast-cells and polymorpho-nuclear leucocytes with ring nuclei/

nuclei. The mast-cells contain innumerable large granules staining metachromatically with P.M.B. The outward form and size of these cells is not constant but may be round or polygonal in adaptation to the neighbouring elements. The nucleus stains very faintly as a clear blue spot among the granules. The mast-cells occur frequently in rows in the loose tissue, between the fat cells and between the muscle fibres. Their relation to the Clasmatocytes of Ranvier has not been made clear. It is usually stated that alcohol fixation is necessary to bring out the granules but I have found them perfectly fixed in my Zenker preparations. Neither H.E. nor I.H. bring out the granules and with these stains the cells appear as large mononucleated cells with a very wide homogeneous cell-body.

Maximow, in the blood of the rabbit finds all transitions between the small lymphocyte and the large mononuclear cells and groups them together as Lymphocytes. As the "wandering cells" in the tissue cannot be distinguished from the blood lymphocyte he gives the very natural explanation of their origin by emigration from the blood. This claim is based on

(1)/

- (1) the morphological similarity between the two cells,
- (2) the original derivation from a primitive "primary wandering cell" (Saxer) in the embryonic connective tissue.
- (3) no evidence being found of the transformation of connective tissue cells into wandering cells.

Hirschfeld<sup>52</sup>\* who has made a careful investigation into the comparative morphology of the leucocytes of the blood, states that in many animals the lymphocytes equal in number the polymorphonucleated cells. I have fully confirmed this in blood films of rabbits' and rats' blood but have found few of the larger mononucleated cells and the transition forms described by Maximow. This large proportion of lymphocytes must be taken into account in considering the cells present on the inflamed area.

(2)/

## (2). HISTORICAL

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It is impossible to give even the shortest resume of the numerous works that have appeared on the histology of inflammation and repair. I have been able, however, to find few articles dealing with the healing of incised wounds but the processes in Inflammation and in Wound-healing run parallel courses and it is unnecessary to separate them.

John Hunter (1790) laid the foundation for the more modern conceptions of wound-healing and his view of healing by Blood Clot remained the dominating one till almost the middle of the nineteenth century.

Schwann in 1838 enunciated his cell-theory and accounted for the new formation of cells by their rise from the plastic exudate.

Virchow in 1858 enunciated the now well-known doctrine "omnis cellula e cellula" and set forth the conception of a series of cell-divisions extending backwards uninterruptedly. Virchow derived all the cells of granulation tissue from the increase of the connective tissue cell.

The/



The re-discovery by Cohnheim in 1867 of the emigration of colourless blood-cells from the vessels changed this conception and for a time the origin of the new tissue was derived solely from these emigrated cells. These two views of Virchow and Cohnheim represent the two extreme views regarding the cells of the inflammatory exudate - origin from the pre-existing tissue cells or from emigrated blood cells.

In 1873 the discovery of the Indirect or Mitotic division of nuclei gave special prominence to the first law of regeneration that the newly formed cells are derived always from cells of the same kind - "Omnis cellula e cellula ejusdem generis"

<sup>18</sup>  
Busse\* (1878) investigated the healing of aseptic human skin wounds and his work may briefly be contrasted with the most recent exhaustive work on the histology of Inflammation - that of Maximow. Busse endeavoured to prove:- (1) that the first approximation of the wound is caused not by an exudate but directly by a fibrinous degeneration and swelling of the connective tissue at the margins of the wound -: (2) that the countless cells appearing during the first two days arose through a reversion of/  
of/

of the intercellular substance into cells and nuclei (Grawitz' "Slumber-cell" theory). The colorless blood cells he excluded because in the examination of thousands of specimens, he was able to trace only one instance of emigration. The connective tissue cells he also excluded because he found no evidence of mitosis in these cells till the third day.

Busse thought that the various cell-forms represent various periods of development of one cell - the first cells formed from the intercellular substance are poly-nuclear, these become mononuclear and then spindle-shaped.

<sup>79, 80, 81.</sup>  
Maximow\*\*\* (1902-1907) induced an aseptic inflammation in rabbits and other animals by the introduction of injurious foreign bodies into the intermuscular connective tissue of the lateral abdominal wall. By means of these he obtained different varieties of cells almost in pure culture (Reinculturen) according to the ease with which they were able to penetrate into the interstices of the celloidin capsules. In the normal subcutaneous tissue I have already stated Maximow found (1) connective tissue cells, (2) round "wandering cells" and (3) clasmatoocytes and clasmatoocyte-like adventitial/



adventitial cells. Maximow sets out with the object of following in the course of the inflammatory process the destiny of the histogenous and haematogenous cells. Amongst haematogenous he classes the "wandering cells", the clasmatocytes, and clasmatocyte-like adventitial cells, which by Pappenheim\* Dominici and others are grouped as histogenous cells.

In the early stages of aseptic inflammation three distinct types of cells were found on the inflamed area:-

- (1) Polymorpho-nuclear leucocytes.
- (2) Fibroblasts - the pre-existing connective tissue cells.
- (3) Polyblasts - the mononucleated amoeboid cells grouped conveniently under one name. Already nineteen hours after the introduction of the foreign body, they were found in great numbers. Maximow, therefore, concludes they cannot have arisen from pre-existing tissue-cells (for there are no signs of mitosis) but must have emigrated from the blood-vessels. They are actively amoeboid and develop progressively from lymphocytes into the large mononucleated forms. A few mononucleated cells emigrate as/

as such. Maximow states that a small portion of his polyblasts are also derived from (1) the "wandering cells", (2) the clasmatocytes and (3) the clasmatocyte-like adventitial cells.

Maximow thus states the function of these cell-forms:-

- (1) The polymorpho-nuclear - the first to appear and prepare the ground for the other forms.
- (2) The fibroblasts - the usual connective tissue cells which early proliferate by mitosis and wander - though later than Leucocytes and Polyblasts - into the interstices of the capsule. They are not phagocytic and their sole function is to form fibrous tissue. A few may in the scar tissue become rounded and like the wandering cells.
- (3) The polyblasts - in the area of inflammation these undergo a series of progressive changes which result in the formation of large amoeboid cells with a highly differentiated centrosome apparatus. Many emigrate into the interstices of the capsule and are found everywhere in the inflamed tissue acting as phagocytes. Maximow looks upon Plasma cells as a specially differentiated group of his Polyblasts. Regarding the/

the fate of the polyblasts, Maximow is very definite. Many perish after their phagocytic function is fulfilled or are removed by the lymph stream laden with the products of their absorption. Others settle in the tissue and are transformed into (1) "wandering cells", (2) clasmatocytes, (3) clasmatocyte-like adventitial cells, and (4) a few may become indistinguishable from fibroblasts.

Maximow thus establishes a complete correspondence between the processes in the embryonic development of connective tissue and the inflammatory new formation of connective tissue.

From the great mesenchyme layer some cells differentiate as connective tissue cells; others as free wandering cells - from which arise the leucocytes of the blood and the wandering cells of the connective tissue. These latter in process of ontogeny become sessile: forming the round wandering cells, the clasmatocytes, and the clasmatocyto-like adventitial cells.

In inflammation the fibroblasts form the great mass of the new tissue. On the other hand amoeboid cells arisen from the blood and to a small extent/

extent from the "wandering cells" of the tissue, the clasmatocytes and the clasmatocyte-like adventitial cells all form polyblasts. After their function is fulfilled those remaining in the tissue become sessile as round "wandering-cells" of the tissue, as clasmatocytes, and clasmatocyte-like adventitial cells.

(3)/

### 3. THE CHANGES WHICH OCCUR IN AN INCISED WOUND LEADING TO ITS COMPLETE HEALING.

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#### INTRODUCTORY NOTE:

For convenience in description I. have referred to certain layers in relation to the depth of the wound.

- (1). The superficial layer - extending from epidermis to the superficial muscle:  
(Plate 26a.).
- (2). The middle-layer - including the loose tissue between the superficial and the deep muscles. In this layer occurred always the greatest reaction.(Plate 26b)
- (3). The deep layer, between the muscle and reaching to the peritoneal surface.  
(Plate 26c.).

These photos were taken from the same wound and in this instance the layers were immediately superimposed on one another. Had this always been the case the interpretation of the changes would have been greatly simplified, and their description/

description rendered more intelligible. Usually the wound edges do not lie in apposition. The severed epithelium of the deeper edge is agglutinated to the opposite wound surface, and the projecting wound edge is bordered by a thin layer of fibrin and is infiltrated with leucocytes. In the tissue the wound is followed by a long undulating line of fibrin; this usually forms a fairly wide irregular fissure which broadens below to a triangular or diagonal space (Plate 26b.). The course of the wound is marked by irregular fragments of connective tissue bundles and muscle fibres.

Again, the extension of the wound has been divided into zones or areas from the centre outwards on each side.

- (1). The central 'fibrin strip'. (Plate 20)
- (2). The 'necrotic zone' of degenerated tissue elements and leucocytes bordering the fibrin. (Plate 22 and 37)  
(edge of photographs.)
- (3). The 'wound area' where the tissue elements have been injured but only sufficiently to stimulate them to increased activity. (Plate 21).
- (4)./



- (4). The 'zone of reaction' extending outwards on each side to normal tissue, and lessening in extent as the intensity of the inflammatory process diminishes. (Plate 23).

STAGE I. (One to eighteen hrs.)

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- Six hours: The space between the wound edges is filled up by the extravasated blood from the cut vessels and the exuded serum. Fibrin very soon forms in consequence of the destruction of the leucocytes and the liberation of the fibrin ferment. This fibrin fills the gap, agglutinates the edges, and fills the interstices of the adjoining tissue. The fibrin when first formed is granular but very soon the granules are arranged into threads which form a network, in the meshes of which numerous red blood cells and leucocytes are enclosed (Plate 20). This figure is taken from a later date but I refer to it here as it is one of the few cases where I was able to get even approximate apposition of the wound edges and it shows the fibrin strip between these.

The necrosed tissue - the result of the trauma/

trauma - and the fibrin together act chemiotactically on the leucocytes and already in six hours there is a streaming of the leucocytes to the injured area. These first-appearing cells are the polymorpho-nuclear leucocytes - they densely border the fibrin and infiltrate the wound-edges. Many have penetrated the fibrin in which red blood cells are still recognisable. (Slide 26) illustrates these points. Allowing for the irregularity in the relation of the parts, we see how the extravasated blood has permeated the tissue at the wound-edges, in some parts fibrin has formed and red cells lie isolated or in groups between intact or degenerating tissue elements. Dilated capillaries and veins filled with blood border the wound. (Plate 21.).

The endothelium of these vessels is already swollen and the margination and emigration of the polymorpho -nuclear leucocytes are very marked. In some vessels the lymphocytes are pressed close against the vessel wall. This is better brought out at a later stage but slide 26 shows two cells, which from their dark nucleus and from comparison with the numerous undoubted pictures of leucocyte emigration (slides <sup>26</sup><sub>27</sub>) I take to be instances of lymphocyte/

lymphocyte emigration. (Plate 5.fig.a.6 hours). The dumb-bell shape and dense structure of the nucleus is quite distinct from the distorted, drawn-out nucleus of the leucocyte: but in many other cases there is very great difficulty in coming to a decision. The tissue cells in the wound area have a pale, scarcely distinguishable outline.

The tissue in the neighbourhood of the wound - the zone of reaction - shows marked oedema. The collagen fibres are swollen, homogeneous, separated from one another, and frequently break up into fibrils at their ends. The vessels are dilated and filled with red cells and leucocytes and a few lymphocytes, lying between the swollen collagen fibres and the dilated vessels are numerous cell-elements.

- (1). The ordinary polymorpho-nuclear leucocytes which are fixed in all stages of active movement and show the greatest variations in form of the nucleus. These cells are both isolated in the tissues and collected in groups around vessels. No signs of degeneration in the cell are seen in this zone. They are evidently newly-emigrated cells on their way to the/

the field of battle - the wound area, where their compatriots are already massed, many of them already slain.

- (2). Round nucleated cells, which would seem to be the ordinary round "wandering cell" of the tissue but that they are gathered in such numbers especially around vessels. A few larger forms with slightly indented nucleus and a broader edge of protoplasm, (staining light blue with P.M.B.<sup>103</sup>) are present:

- (3). Cells which are at once recognised as the connective tissue cells. They are distinctly swollen and separated from one another and from the collagen fibre by the oedema. The cell body is more defined and stains with P.M.B. more darkly, and with I.H. has a more granular and reticular character: but otherwise the cell in this zone is little changed. Amongst these cells there must be some which are to be numbered among the endothelial cells of lymphatics and of the tissue/

tissue spaces - if such are lined with endothelium.

In none of my specimens have I seen any evidence of amitosis such as Kiener and Duclert found in the early stages of suppurative inflammation. Nor is there any evidence of Mitosis in any of the cells and little change in the few adventitial cells.

Already then at six hours we find:--

- (1). Formation of fibrin between the wound-edges and tissue interstices.
- (2). Accumulation of leucocytes and degeneration of tissue elements at wound margin.
- (3). Dilated vessels, with margination and emigration of leucocytes and probably lymphocytes, in the wound area.
- (4). A swelling of all the tissue elements in the zone of reaction and, distributed in the widened meshes three kinds of cells - leucocytes, mononucleated cells, and connective tissue cells.

Twelve to Eighteen hours: The fibrin has now become distinctly fibrillar on the borders adjoining the/

the tissue but has a more open network in the interior. (Slide 228.) Dense masses of leucocytes and chromatin particles, together with degenerated tissue elements, border the fibrin strip.

In the wound area the vessels are still more dilated, the grouping of the leucocytes inside and outside the vessels and in their walls is more marked. In these dilated vessels the margination of the leucocytes and the flattening of the lymphocytes against the wall is very evident. Numerous red blood cells are found in the vessels and in the tissues. In many of the vessels in this area a granular eosine-stained (H.E.) deposit is found (Slide 5a). This may be grouping of blood-plates in relation to the thrombosis of the vessels. The endothelial cells of the vessels are very swollen and granular and project into the lumen almost closing it.

In the zone of reaction reaching on either side of the wound for a considerable distance according to the intensity of the trauma, the whole capillary meshwork is made evident. Around these dilated vessels are numerous leucocytes and mono-nucleated cells, the latter increasing in proportion as we near/



near the normal tissue. These mono-nucleated cells are now becoming larger in size, the protoplasm more reticular. The cell-body stains more violet with P.M.B., and with H.E. has a purplish tinge instead of the eosine-stained border of the smaller cells. The cells have been fixed in amoeboid movement and, especially the larger forms, show irregular, serrated projections of different form and size. The nucleus becomes indented and clearer in structure. The increasing indentation of the nucleus has been explained beautifully by Gulland\*<sup>40</sup> as the effort of the cell during its progressive development to find its Kinetic centre. In this indentation a clear area is becoming evident in the cell body as the cell enlarges. The nucleus shows with I.H. one or two larger chromatin particles which with M.G.P. stain with the pyronin, thus showing that during its enlargement this cell develops true nucleoli. This is not very evident at this stage but at later stages the smaller and larger forms show this difference very clearly. This has been brought out very beautifully in Plate I. (a) where in the fibrin layer on the surface of a wound different sizes of mononucleated cells are found, the larger only with nucleoli. As the cell increases in size, the protoplasm/

protoplasm takes a deeper shade of pink with M.G.P. but is frequently almost colorless. Further stages in the development of these mononucleated cells will be discussed in later sections. In the rat especially, many of these cells are becoming vacuolated and I have in one or two cases found enclosed red-cells (Slide <sup>229</sup><sub>234</sub>) showing that these cells have already commenced that phagocytic activity which develops so greatly during the next stage.

The connective tissue cells in a wide area are enlarged the protoplasm is more distinct and more granular; the cell-processes are more definite. These branching swollen ends give the oedematous tissue the appearance of mucous tissue with swollen dissolving remains of the collagenous fibres. (Slide <sup>229</sup>).

Plate 6. (fig.b.18 hours) shows the proportion of mononucleated cells to the polymorphonuclear around dilated vessels in the zone of reaction farthest removed from the wound: and at the same time shows the morphological similarity between these mononucleated cells and those found within the vessels. In this region the mononucleated cells have not yet progressively developed - this takes place/

place the nearer we come to the wound area where their work is to be done.

(Slide 6.) from which this drawing is taken shows at many points the swollen endothelial vessel cells and the enlarged connective tissue cells. In both of these cell-forms a re-arrangement of the chromatin loops can almost be made out especially with I.H. (Slide 80) in preparation for division.

A very exhaustive examination of very many sections from wounds at all stages between twelve and eighteen hours both in the rabbit and rat, has given me no single instance of undoubted mitosis in either connective tissue or endothelial cells. It is acknowledged by all writers that in the tissues any evidence of mitosis in the fixed cells is not found before twenty hours. Maximow chooses as the first stage of his examination of aseptic inflammation nineteen hours "because in the course of the first nineteen hours the possibility of a multiplication of pre-existing tissue cells can be absolutely excluded".

#### SUMMARY OF STAGE I.

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During this stage up to eighteen hours, which in my introduction I have named the stage of the/

the vascular phenomena, we find:-

- (1). an increasing dilatation of the vessels with the phenomena we associate with this:-
- (2). already a commencing phagocytic activity of the mononucleated cells, and
- (3). already a response on the part of the fixed tissue cells - the connective tissue cells and the endothelial - which show enlargement and granularity of the protoplasm.

The cells found at this period on the inflamed area are of three distinct types:-

1. The polymorpho-nuclear leucocyte whose origin is clearly emigration from the blood .
2. The connective tissue and endothelial cells - the pre-existing fixed tissue cells.
3. The mononucleated cells of varying size and shape, from the small lymphocyte-like cell with narrow border of protoplasm/

protoplasm and almost no nuclear structure to the larger mononucleated cell with indented nucleus of clearer structure and true nucleolus. This group gives us the so-called "Mononucleated cells of the Inflammatory Exudate" in regard to whose origin, function, and destiny so much research has been done.

#### STAGE II. (Eighteen to Thirty hours).

We have seen that every injury, however small, causes a certain amount of tissue damage. Before repair can be effected by the proliferated tissue cells this damaged material must be removed. Together with the degenerated local tissue elements we have numerous dead leucocytes, extravasated red blood cells, and a mass of granular or thready fibrin all of which must be absorbed ere complete restitution can occur. Maximow, as a result of his investigations on the histology of aseptic inflammation, concludes that the first cells called out in the defence of the organism are the polymorpho-nuclear leucocytes: that these in some way prepare the ground for/

for the mononucleated cells but are themselves destroyed in large numbers in the process. The mononucleated cells then exert their marked phagocytic power absorbing by an intracellular and an extracellular activity the remains of tissue elements, cells, and fibrin. Thus the ground is prepared for the connective tissue.

It is this period of phagocytic activity and preparation I have taken for my second stage. In my preparations only a short period intervenes between the time I have first noted definite phagocytosis by mononucleated cells and the period of a marked proliferative activity of the connective tissue cells with a streaming of these 'awakened' fibroblasts to the seat of injury. The two processes of phagocytosis and tissue cell-reaction go on side by side, the former gradually lessening as the work is accomplished, the latter resulting in the production of an abundant capillary network (which assists in the absorption of dissolved decay products) with numerous fibroblasts in the meshes.

A typical wound-fissure at this stage would be represented by a more or less broad, irregular, strip of fibrin: the centre of this is still granular/



granular or is a thready meshwork with a few leucocytes and still recognisable red blood cells and possibly the remains of degenerated connective tissue cells. The borders stain deeply in consequence of the numbers of leucocytes and degenerated chromatin particles. This is the fibrin strip and necrotic zone, outside of which is the zone I have called the "wound-area" - where the tissue elements are still recognisable and the dilated vessels are surrounded by very numerous leucocytes, mononucleated cells, and a few reacting connective tissue cells. It is in this zone those absorptive processes occur which will prepare the way for the fibroblasts.

Amongst the dilated capillaries in this zone we get very numerous mitoses already in twenty-one hours, marked in thirty hours. The remaining connective tissue bundles in this area are swollen and homogeneous but can be distinguished from the fibrin by the van Gieson stain. Their appearance led Busse and earlier investigators to ascribe the first adherence of the wound-edges to a fibrinoid degeneration of the connective tissue.

Farther out we come to what I have called the "zone of reaction" which extends on either side for/

for a distance proportionate to the intensity of the injury. In this area are dilated vessels surrounded by cells which are to aid those in the wound area and, distributed in the tissue are the awakened connective tissue cells, some of them already proliferating, others preparing for their work on what has figuratively been spoken of as the field of battle. Their time has not yet come but where the destruction has not been very great, and the stimulus probably just sufficient to incite these cells to proliferation, we get already before thirty hours very numerous mitoses. In the loose tissue between the superficial and deep muscles we see most strikingly this appearance of the fibroblasts and of the vessels surrounded by numerous mononucleated cells. In this region too, already, we get dilated lymphatics containing mononucleated cells laden with decay products returning from the wound area.

Plate 20. (wound 24 hours) represents a wound-fissure with central fibrin strip and necrotic zone with leucocytes. (The reaction in this case was not very intense but for reasons already given this preparation was photographed).

Plate 21.<sup>21</sup> (wound 21 hours) represents the /

the reaction in the wound area and brings in much better the necrotic zone, numerous very dilated capillaries, and leucocytes. In slide 21 it is seen that amongst these are mononucleated phagocytic cells.

Plate ~~18~~<sup>22</sup>. (wound 24 hours) shows in the wound area corresponding to Plate <sup>21</sup>22. capillaries almost closed with proliferated endothelial cells. In one of these two cells are found in mitosis.

Plate 23. (wound 21 hours) shows the reaction zone with dilated vessels and numerous cells especially in the still oedematous loose layer.

Plate 3 (wound 24 hours M.G.P.) - a group of dilated capillaries in the wound area. The granularity of the swollen endothelial cells well brought out by the pyronin. In two of these capillaries endothelial mitosis is found.

Plate 5 (fig.a.) shows a portion of a larger vein at the very margin of a wound (21 hours) in longitudinal section. The vessel wall is penetrated by leucocytes in all stages of emigration through it and surrounding the vein are numerous similar leucocytes fixed in all stages of amoeboid movement. Numerous mononucleated cells are also found outside the vessel.

Very/

Very numerous additional examples of these changes are seen in slides from 21, 24, 27 and 30 hours. Slide 5a shows the dilated vessels at the wound margins with numerous cells in their walls. Definite endothelial mitosis is present in many and, in others, cells are found, with a denser and broader nucleus than that of the leucocyte, passing through the vessel wall often in a slanting direction. These may be emigrating lymphocytes but, remembering how many of the vessels are blocked with proliferated endothelial cells and the migratory power of these, one feels bound to conclude that these are endothelial cells passing out into the tissues.

Pappenheim<sup>101</sup>\* has objected to Maximow's pictures of lymphocyte emigration, stating that they are much more likely to be endothelial cells passing through the vessel wall. This supports the view that these cells are emigrating endothelial cells.

These appearances are found specially abundantly in the vessels of the wound area (Plates 3 and 18). The mitotic figures in Plate 3 give one the impression that the resulting cells are becoming free in the lumen, whence they may migrate out into the tissue. In other cases the resulting cells seem/

seem to form a double layer of cells at one part, whence one may simply pass out into the tissue: both appearances can be made out. In one vessel the mitotic figure - a dyaster - showed the long axis of the parent cell at right angles to the vessel wall instead of the usual arrangement parallel to it. This may have been accidental but it is of interest in view of the statement above that these vessels are filled with proliferated endothelial cells and of the possible origin of the mononucleated blood cell from blood-vessel endothelium.

(Adami)\*. Here it seemed as if one resultant cell would be set free in the lumen, the other remains attached as the lining cell. Slides 234 - 239 all show instances of proliferation of endothelium, and also an increase of the cells attached to the vessel-wall outside - a possible adventitial cell-proliferation. It is extremely difficult to satisfy oneself about the proliferation of the adventitial cells. In the normal tissue they are so small and undeveloped and in inflamed tissue so many cells surround the vessel wall. The possible origin of these may be:-

- (1).. a simple passing outwards of the outer  
of two endothelial cells after mitosis.

- (2)./

- (2). an emigration of endothelial cells.
- (3). an increase of the adventitial cells.
- (4). emigrated mononucleated cells as in the early stages.

The object of the stress laid here on the intense proliferation of the endothelium and its possible migration is to support the view of Mallory and Beattie that from the endothelium are derived many of the mononucleated cells. It is at this time one finds the mononucleated cell in greatest numbers in a wound not accompanied by too great inflammatory reaction. In other tissues the endothelial cell is found to be actively phagocytic and it is at this period - before the active fibroblast proliferation begins - that a reinforcement of mononucleated cells is necessary. The cells resulting from the division of endothelial cells have probably the same characters as the larger forms of mononucleated cells with a clearer, frequently indented nucleus and a nucleolus which stains with pyronin. Around the vessels in Plate 3 are cells which by an unbiassed observer must, I think be acknowledged to be those which we associate with the term "mononucleated/



"mononucleated cells of the inflammatory exudate". In the vessels are mitotic cells becoming loose which one can quite believe are the sources of these cells outside. The number of cells actually phagocytic at this time is small compared to the large number in the tissue. Many show extensive vacuolation which is probably a sign of increased activity and may have to do with their extracellular action.

In the zone of reaction are still found leucocytes and lymphocytes fixed in all stages of movement: the latter often with nucleus gourd-shaped and dense in staining. At this period I found much more abundantly than at other times the polymorpho-nuclear leucocyte nuclei assume rosette forms which were occasionally difficult to distinguish from mitoses.

Connective tissue cell proliferation has also commenced but is by no means so marked as the endothelial mitosis. Slide 235 (21 hours) shows two connective tissue cells in the subperitoneal tissue in mitosis. This was the earliest date on which I found mitosis in either connective tissue cell, or endothelial. In the period from 18 to 30 hours, the endothelial mitosis was at least three times as/

as numerous as in the connective tissue cell. The proliferation of the connective tissue cell results in cells to which Ziegler has given the name fibroblast because they are the formative cell of the fibrous tissue. Almost all authorities are agreed that the descendants of the connective tissue cells are at first small round cells indistinguishable from lymphocytes. Maximow, in numerous parts of his work, states distinctly that this is not so - basing his assertion on the character of the fibroblast nucleus with its nucleolus and the more defined contour of the round cell body. I have frequently found evidence to support Maximow's view and think that it clears away at least one of the many difficulties which beset this study. On the inflamed area we have three distinct types of cells:-

- (1). Leucocytes - with a definite origin and function.
- (2). Mononucleated cells - presenting a great variety of forms - which has led Maximow to give these cells the name Polyblasts - and derived from a great many sources: yet in spite of their manifold origin have all a function related/

related to both their intra - and extra-cellular activity.

- (3). Fibroblasts - with a definite origin and function, recognisable at once as descendants of connective tissue cells. If, however, at one stage of their career, the fibroblasts are like the small mononucleated cells we cannot state if it is only the descendants of the connective tissue cells that give rise to cells forming fibrous tissue.

STAGE/

### STAGE III. (Thirty hours to Four days)

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The previous stage was related to the reinforcement of the mononucleated cells chiefly by the proliferation of the endothelial cells. This one is concerned with the carrying out of their function and with the active proliferation of both connective tissue cells and endothelium - the reaction of the tissue elements to regain lost ground. The endothelial proliferation is now to be referred rather to the new formation of blood-vessels than to the production of phagocytic cells. Plates 18 and 22 show the endothelial reaction referred to in the last stage. The vessels in which these changes are occurring are in the wound area, closely surrounded by fibrin and leucocytes - showing pyknosis and karyorrhexis - and the products of their disintegration. It is in this region that more phagocytic cells are needed and in the zone of reaction in a wound running a normal course I have not seen vessels filled with swollen cells which convey the same impression. It is not from the vessels that immediately border the necrotic zone that the young buds arise which penetrate the fibrin - an indication of this is/

is given in Slide 25 from which Plate 25 was taken.

Thirty hours to Two days:- In the fibrin which still occupies the wound cleft we find an increasing number of cells, which have passed the barrier of necrosis at the edge of the fibrin. Amongst these are phagocytic mononucleated cells, many extremely vacuolated and others containing red-cells and leucocytes: also a few star-shaped fibroblasts with long processes. At the margin of the fibrin still lie chromatin masses from the disintegrated leucocytes but this border is becoming broken up. Numerous mononucleated cells penetrate through it absorbing and dissolving its substance.

In the wound area the vessels are still dilated, but the number of leucocytes surrounding them is greatly diminished; numerous phagocytic cells are present between the vessels; the small capillaries which earlier gave the impression of being blocked with cells are opened or show a swelling on one side.

Not only the vessels at the wound margin but those which border the zone of reaction show a greatly increased capillary meshwork. This arises from a production of new vessels which are very small in section. These have usually a nucleus at one side/

side giving it a signet-ring appearance. Numerous mitoses may be found in all the sections for this period. Slides <sup>240-242</sup> 242, 244, 247, 211, 212.

The proliferation of the connective tissue cells is now very evident. Slide 240 shows three such cells in one field in mitosis. Slides <sup>240-247</sup> 240-247, 211, 212.

all show very numerous mitoses. It is in the loose layer on either side of the remains of the superficial muscle that the fibroblasts are found in greatest numbers. The young fibroblasts are mostly elongated cells with their long axes all radiating in one direction - the focus of attraction being the wound-fissure. Several reach and penetrate the fibrin where we have seen them as star-shaped cells. Probably as long as they need to migrate these fibroblasts are elongated oval spindles with a process at either end but when they reach their goal, they assume the most varied forms but most frequently are star-shaped with processes arising from all sides. Maximow thinks they have used the fibrin threads in the tissues as guides. The protoplasmic processes of these young fibroblasts are very beautifully brought out with M.G.P. and with I.H. the finer structure of the nucleus with numerous very fine dust-like/



dust-like chromatin particles connected by linin threads is made very evident. Mitoses are found chiefly in the zone of reaction a little removed from the wound-border and the resultant fibroblasts stream as it were to the wound-fissure. This "awakening" of the fibroblast gives the impression of a definite purpose and together with the increased capillary network indicates that the tissue reaction has commenced in earnest. Slides 243 and 245 from a wound of thirty hours show especially well this streaming of the fibroblasts.

In the loose layer are found numerous phagocytic cells and leucocytes. Some of the former may be mistaken for the young capillaries of signet-ring shape if the cell-inclusion has become digested leaving a large vacuole which presses the nucleus to one side. In the larger mononucleated cells a clear area is becoming differentiated opposite the indented nucleus. This clear hof with contained centrosome is often surrounded by a ring of granules (I.H.)

The old collagen fibres near the wound area are at many parts almost dissolved and their place occupied by this network of capillaries, streaming fibroblasts and mononucleated cells. In the widened lymphatics we find numerous cells containing cell-debris/

cell-debris and many vacuoles.

Two to Four days: The same three phenomena which marked the earlier part of this stage are now present in a more intense degree. The absorptive process is being actively carried out by the phagocytes, the new vessel formation is very marked; and the connective tissue cell proliferation results in an enormous increase in the number of the cells. In the more aseptic wounds the vascularity and the leucocyte emigration are diminishing and in a small wound the epithelium may have covered the surface.

The fibrin still remains often in considerable quantity but in the meshwork in the interior are very numerous cells - fibroblasts - with broad-based, branching, anastomosing processes, and phagocytic cells with cell-inclusions or many vacuoles.

The necrotic zone bordering the fibrin has in four days practically disappeared. (Slide 25 ) The dead leucocytes and other cell and tissue debris have been taken up by the phagocytes, or their dissolved products have been removed in the lymph and blood stream. Numerous young vessels penetrate this zone (Plate 25) which is now replaced by phagocytic cells, fibroblasts, and interlacing vessels penetrating/

penetrating the fibrin. Masses of dead leucocytes and chromatin particles are still found - remains of the necrotic wall that bordered the fibrin and the tissue. The fibroblasts are elongated cells with branching processes and are arranged radiating towards the fissure from all parts of the wound area.

The wound area which can no longer be distinguished from the necrotic zone contains the same young vessels and fibroblasts and mononucleated cells.

In the reaction zone there has been very abundant production of fibroblasts of an oval, spindle form which are again arranged radially. Around the old vessels a capillary meshwork has arisen in consequence of the numerous "buds" from the vessels (Plate 18). Between these groups of young capillaries the cell tissue is permeated with cells:- fibroblasts, arranged with their long axes pointing to the wound, mononucleated cells, and a diminishing number of leucocytes.

The fibroblasts . At this period these are large, beautiful, spindle-shaped cells, with a process at either end which gives them the appearance of being able to penetrate readily. Later they become branching and as they reach their destination they/

they become even star-shaped, or spear-shaped. The processes interlace with the processes of adjoining cells: and frequently form a dense plexus of cells in which almost no vessels can be discovered.

(Slide 250<sup>2</sup>). When the fibroblasts have replaced fat tissue this plexus formation is specially noticeable (Slides 76.36.248). The structure of the cell can now be made out very clearly with numerous stains, especially when the cell lies among the fibrin and is submitted to no pressure. Here the granular appearance of the reticular protoplasm is brought about beautifully by M.G.P. which also stains the projections to their finest endings.<sup>(285-286)</sup> The nuclear network is not so evident as with (I.H.) but several pyronin-stained nucleoli stand out as the most characteristic features of the nucleus. I.H. brings out the beautiful linin network, the fine chromatin dust particles, and the dark-stained nucleoli. I have frequently recognised the centrosome apparatus in these large young cells. (75.213-214)

THE MONONUCLEATED CELLS. Those in the fibrin and wound area are nearly all phagocytic. Lying in clear spaces in the cell-protoplasm are fragments of cells and chromatin particles, and many show only a mass of vacuoles. Sometimes these vacuoles/

vacuoles stain very purple with M.G.P. and a mulberry-like cell is found especially in the fibrin strip (Slide 62. ) which has every sign of being a degenerating cell. These phagocytic cells are found not only in this area but in some preparations are very numerous in the loose layer in the reaction zone: some of these are evidently returning in the lymphatics. Slide 87 shows a dilated lymphatic space filled with such cells and cell-debris.

Numerous mononucleated cells are found in the zone of reaction showing all transitions in size from the small lymphocyte to the larger forms which are becoming phagocytic. As they increase in size the development of the nucleolus is very characteristic. Maximow considers this a late development of the cell and that the want of it is one of its chief differences from the fibroblast cell. In my preparations many cells showing only a slight increase in size and slight indentation of the nucleus show a well-marked nucleolus. The larger forms may be mistaken for cross-sections of fibroblasts, but in the latter several nucleoli develop and the whole framework of the nucleus is clearer. There is no difficulty in coming to a decision in regard/

regard to most of the cells. The indentation of the nucleus develops progressively with the clear unstained "hof". In this "hof" with I.H. very definite centrosomes can be found. The cell membrane is frequently folded to form almost a complete partition in the nucleus. The protoplasm is reticular and frequently vacuolated especially as the cells near the wound area. Where there are no inclusions and no vacuoles the protoplasm stains a more intense red with the pyronin as the cell enlarges; and cell-forms are found which show a great similarity to the fibroblast with its intensely stained pyronin protoplasm. Slight differences such as the denser structure of the nucleus, the presence of only one nucleolus, the irregular projections from the cell instead of cell-processes, mark off the two cell-forms.

Maximow has described a characteristic difference in the appearance in mitosis. In the fibroblast, the reticular structure of a dividing cell is stained intensely and the nuclear figure occupies a transparent halo in the centre of the cell. In the mononucleated cell the clear area enclosing the nuclear figure is wanting. The presence of this halo is very marked in some of my preparations. e.g. Slide/



Slide 247. (wound 3 days ) shows in one field five cells in mitosis which under the high power, all prove to have a halo. The absence of the halo has not, however, been at all convincing yet in a few instances (Slides 62 and 132.) with M.G.P. the nuclear figure was in a diffusely-stained area and the cell gave the impression of being a mononucleated one. Mitosis in these cells is however, very limited, but occurs even in intensely phagocytic cells. (Plate 13.)

Borst<sup>15</sup> believes that the law of the specificity of cells must find morphological expression in a distinction during their mitotic division. He found that the differences depend on the size and form of the chromosomes and the appearance of the spindle figure. Thus he distinguishes the endothelial and connective-tissue-cell mitoses by the extreme delicacy of the chromosomes. My work has given me no indication of these differences of the endothelial figure, but different fixatives might not have limited the usefulness of my material in regard to this question.

THE BLOOD-VESSELS. New formation of blood-vessels has been taking place very actively. This subject must be discussed later and I only briefly refer/

refer to the appearance during this stage. Slide 260 shows a beautiful capillary network occupying the whole area where the fibrin had been. Many of the endothelial cells are in mitosis and are surrounded by phagocytic cells. In numerous preparation groups of vessels on cross-section are surrounded by groups of capillaries showing a signet-ring appearance. Slide 140 and in other parts (Plates 4 and 18) the vessels show a great increase of cells at one side. The contrast between the earlier proliferation in the blood-vessels, which resulted in the loosening of the endothelial cells into the lumen and the later proliferation which results in new vessels is clearly brought out by comparing Plates 3 and 22 with Plates 4 and 18: the latter of which only I look upon as having the significance of budding capillaries.

SUMMARY: We have now reached the formation of a vascular and cellular tissue which almost completely replaces the fibrin. This tissue has arisen as a result of the reaction of the blood-vessel endothelium and connective tissue cells. To this young tissue, composed of blood-vessels and numerous cells, the name of "granulation tissue" has been given/

given - a name applied now to all cellular proliferations including those of the granuloma. As the fibrin was only a temporary formation for the purpose of bridging the gap, so this mass of granulation tissue is only a temporary framework by means of which the firm tissue will be built up. The majority of the young vessels will be obliterated: many of the cells when their function is fulfilled will be removed: and a fibrillated tissue will be laid down.

STAGE/

#### STAGE IV. Five to Seven Days.

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In the former stage we saw that the leucocytes had considerably diminished in number, except around areas of special irritation e.g. the stitches. The phagocytosis too had lessened and the sway of the proliferating tissue cells - connective tissue and endothelial - was in full force. In this stage the connective tissue cells take the chief part in the evolution of the new tissue. They come to form parallel layers between the blood-vessels and, by their further differentiation, a young scar tissue is produced. The essential feature is the re-arrangement and transformation of the structural elements which are to remain permanent.

Small wounds are frequently healed by this time but this description has been taken from those of my wounds accompanied by acute inflammatory reaction yet where the healing process has been progressive.

Slide 253 (wound 6 days) shows a wound healed over by epidermis. The sub-epidermal capillary network at the margins of the wound is very evident, but immediately under the new epidermis elongated/

elongated fibroblasts lie parallel to the surface. A little deeper these cells are arranged more irregularly but radiating towards the centre, and amongst them are found remains of collagen bundles and fibrin which have resisted dissolution. Delicate capillary loops penetrate amongst these elements and an attempt is being made at a gradual arrangement of the cells parallel to the vessels.

In the deeper layer the fibroblasts are elongated cells with several anastomosing processes thus forming a plexus of gradually increasing density. In the protoplasm of these cells we find commencing fibrillation. Numerous fine fibrils in the cell-protoplasm are recognised by means of both the van Gieson and Mallory's stain. These fine fibrils seem to leave the cell by means of the processes which frequently break up into almost a ray of fibrils.

The leucocytes are very scanty in this layer and are very irregularly distributed, never grouped together into small heaps.

The mononucleated cells are also very few in number in the superficial layer. They are now more definitely sessile cells, which have given up their phagocytic function and are recognised by the denser structure and the indentation of the nucleus. They/

They are found chiefly close to the vessels or in the meshes between the fibroblasts.

Near the triangular space in the middle layer, where remains of fibrin and coagulated blood still remain numerous mononucleated cells are still to be found. Here they are very phagocytic. When there are numerous cell-inclusions in the cell its borders are frequently difficult to define but near the nucleus the protoplasm structure is always reticular. The blood-vessels are still wide loops, with walls of a single layer of endothelial cells and arranged just outside them are cells which on longitudinal section of a vessel cannot be distinguished from the surrounding connective tissue cells. Between these two layers of cells is frequently found a cell which can at once be recognised as a mononucleated cell with an oval, dark nucleus and drawn-out cell body. These are the sessile become mononucleated cells whose function is probably that of forming an adventitia to the vessel, and which correspond to Maximow's elasmatoocyte-like adventitial cell. In the protoplasm of these cells in wounds I have never been able to trace any granules but in the abscess membrane at a later date, specimens stained/



stained with I.H. frequently show granules at each pole of the nucleus.

In the triangular space in the middle layer the process of healing is not nearly so advanced: around the remaining fibrin are gathered numerous mononucleated cells and young capillaries which send into it beautiful young shoots and buds.

In the deeper layer next the peritoneum the process is again much more advanced. The gap in the muscle wound has been filled by a cellular tissue composed chiefly of elongated fibroblasts, which show a much more marked fibril development than in the superficial layer. The mass of new tissue in this layer has the appearance of a wedge with its base towards the peritoneal surface. From the angles of the base the fibroblasts, curving inwards, stream in such closely applied rows that there seems no room for blood-vessels or other cells. On the peritoneal edge two or three layers of darkly-stained oval cells are found and a gradual transition in length and character can be traced between these and the cells passing inwards (Plate 27). This appearance was so frequently seen (slides 27.28, 28.3, and numerous others) that it suggested that these cells  
on/

on the peritoneal edge were the same cell-forms as the streaming fibroblasts. Numerous fallacies might arise here - the peritoneal endothelium had probably degenerated and disappeared for a considerable distance from the wound edges and had not yet been replaced or, again, the condensation at the edge might have been artificial.

In all my specimens there was such a marked proliferation of fibroblasts from the sub-peritoneal layers of connective tissue that the conclusion was forced on me that some unexplained stimulus was acting here. In numerous specimens mononucleated cells were found in all transition forms to the peritoneal endothelium, a little removed from the wound and just here had commenced that streaming-in of the fibroblasts which resulted in a scar tissue so much further advanced than in other parts of the wound. If the mononucleated cells are unicellular glands, as so many suppose, and if they can arise by proliferation from endothelial cells, might not their extracellular action be the stimulus which caused this active proliferation? (Wade<sup>135</sup>).

The relation of endothelium to fibroblast formation, however, will be taken up in a later section/

section.

Plates 25a..25b. 25c. illustrate the three layers of a laparotomy wound at this date in which the healing process has been delayed and a very cellular infiltration of the superficial layer is present. Considerable irritation has drawn forth large numbers of leucocytes which, together with the fibrin, have formed a crust on the surface of the wound. The epithelium is seen stretching under this crust and is found in islets among the leucocytes and fibrin. The further progress of this wound will be parallel to that described under "The Structure of Granulation Tissue".

Plate 25b. shows in the centre layer a dense coagulum surrounded by fibrin. This seems to illustrate the importance of drainage in a wound, and how delayed is the process of healing at this part in consequence of this clot.

Plate 25c. gives the deep layer and again shows how much more advanced is the proliferative reaction near the peritoneum.

The former wound-cleft is now filled up by

a/

a new tissue in which the great mass of elongated fibroblasts, arranged parallel to the vessels, is the striking appearance. These cells in some way are laying down in the spaces between them the delicate fibrils which in the next stage will lead to the establishment of the tissue.

STAGE/

# STAGE V. Eight to Fifteen days.

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Cicatrization may now be said to be taking place in a more or less aseptic wound. In my incisions which passed through the whole thickness of the abdominal wall, the superficial layer had frequently completely healed while the deeper layers, where there had been very considerable necrosis of muscle tissue, were much later in healing. Stitches too, caused great differences in the period of healing. Sometimes they caused comparatively little reaction - being early encapsuled by layers of leucocytes. At other times they caused extensive tissue necrosis and leucocyte infiltration.

I have chosen out of numerous examples of wounds at this stage three of nine, eleven, and thirteen days. In the first the subcutaneous tissue was replaced by layers of fibroblasts parallel to the surface with delicate fibrils between the cells; in the second, the wound which was a small one - though skin and subcutaneous tissue - had completely healed (Plate 29); in the third fibrous laminae were laid down between the cells. Changes in the muscle tissue/

tissue will be considered in a later section, and the reaction of the peritoneum and the sub-endothelial layers.

Slide 254. (Wound 9 days ) shows a broad band of new tissue consisting entirely of elongated fibroblasts parallel to the surface. The sub-epidermal plexus of vessels is very marked and the cells of the Stratum germinativum send processes downwards to join the endothelial cells of the vessels. Between the elongated fibroblasts numerous, wavy delicate fibrils run. Very few mononucleated cells are found in the spaces left between the fibrils and the cells. At the margins of the wound around the vessels are numerous small round cells. Slide 29. (eleven days) shows a very irregular arrangement of the cells in the scar tissue. Numerous mononucleated cells surround the vessels and are scattered among the fibroblasts.

Slide 30 (13 days) shows already a very marked condensation of the fibrous laminae between the fibroblasts. In this wound there has been much greater reaction than in the two former. The fibroblasts in some parts form a dense plexus among which capillaries run towards the surface. Numerous mononucleated/



mononucleated cells surround the vessels and under high power those on longitudinal section are seen to have alongside of them elongated cells in form very similar to the fibroblasts but with a darker nucleus and a more defined cell border. These are the mononucleated cells which are becoming sessile as adventitial cells of the vessels.

In this preparation are seen for the first time in the rabbit isolated examples of Plasma cells. These in many of their characters closely resemble the mononucleated cells before the latter have become phagocytic. In wounds with very little reaction such as Slide 270 (6 days) and Slide 277 (2 days) and Slide 271 (11 days) show, these characteristic cells are absent: they appear here for the first time in a healing wound in the rabbit where there has been a considerable loss of tissue. In wounds of later dates also with excessive reaction they are present in increasing numbers, grouped especially along the blood-vessels lying alongside the sessile-becoming mononucleated cells. This cell will be more carefully studied when dealing with the formation of an abscess-membrane.

In the scar tissue in this wound is seen

a/

a long row of giant-cells running from the depth of the scar towards the surface (Plate 30). These have formed probably around hairs which have fallen into the wound fissure. The presence of giant-cells in granulation tissue is a very constant phenomenon: their possible mode of origin will be discussed later.

We have now reached the laying down of a permanent structure - the fibrous laminae. In new scars the cells are still large flattened, or spindle-shaped elements with thin fibrillar substance between them increasing in density. The vessels, except in the depths of the wound, are still wide and branching with their walls supported by the cells of the scar tissue. The endothelium frequently seems double and a distinct space separates the two rows of cells from the nearest fibroblasts which lie alongside. In this space we find adventitial cells and frequently small round cells. In the deeper layers the vessel is surrounded by many cells, chiefly of the nature of mononucleated cells and the fibroblasts run at right angles to the vessel wall. As the fibrous laminae are laid down the vessels dwindle in size from pressure and are represented in the/

the deeper layers of the tissue by endothelial cords.  
(Slides <sup>182.</sup> 184. and 185. )

LATER STAGES: For a long time the site of the original incision is recognised, even in a small wound, by its cellular character. Gradually, however, the fibrous laminae become condensed, the blood vessels obliterated, and the fibroblasts form small spindle-shaped connective tissue cells attached to the collagen bundles. The remaining mononucleated cells settle in the tissues as "wandering-cells" and as adventitial cells. In the neighbourhood of the vessels in the deeper tissues, the so-called "small-celled infiltrations" arise, especially in wounds which have taken long to heal. The scar tissue becomes much closer than before, and broadens out on the surface while it diminishes in depth below so that many old scars are wedge-shaped with the base to the surface.

Preparations from wounds <sup>42.</sup> 15, <sup>171.</sup> 21, <sup>274.</sup> 22, 31, <sup>266.</sup> 267, <sup>269.</sup> 50, 55, and 70 days have been taken to illustrate these points. My oldest wounds are two from human incised tissues - one, <sup>169.</sup> 5 months; the other <sup>170.</sup> 7½ : both were wounds that had taken a long time to heal.  
Both/

Both show very closely compressed fibrous bundles and few cell elements, except around the tissues where very marked perivascular infiltrations occur. The glands and hair follicles are absent in the scar tissue and the papillae are very imperfectly developed.

STAGE/

## THE CELLS OF THE INFLAMMATORY EXUDATE.

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In approaching this study it is well to remember at the outset that we are dealing with cells which have all been derived by a differentiation of the cells of the mesenchyme. This leads us to expect that on the inflamed area we find -

- (1). cells which have reverted to their primitive developmental type and can therefore not be distinguished from one another, and
- (2). that a mutual substitution of one kind of cell for another or a merging of one kind into the other may take place. Yet on the whole we recognise distinct cell-types and if, as is the case, certain cell-forms closely approximate to others in their final evolution, this does not affect the more or less specificity of each cell-type. Further, difficulties in the treatment of this subject arise from the tinctorial differences in cells due to their functional activity and degenerative/

degenerative changes and again, that the daughter-cells arising as a result of the proliferation of parent-cells are all so much alike.

Only the briefest outline may be given of this great subject.

1./



# 1. THE POLYMORPHO-NUCLEAR LEUCOCYTE:

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These are always the first cells to appear in inflammation. They are actively phagocytic for bacteria and can also take up red blood corpuscles and foreign particles. They respond readily to chemiotactic and thigmotactic influences. The cells take up the bacteria immediately after their escape from the vessels (Plate 45(b)). Many facts point strongly to the production of a bactericidal substance by the leucocytes through a process either of secretion (Buchner) or disintegration (Metchnikoff). Others hold that this substance is free in the serum and has nothing to do with the cells. Wright has named this substance present in the serum "opsonin" i.e., a substance which prepares the bacteria as a food for the cells.

The significance of the multilobed nature of the nucleus has been variously construed. Some see in it a preparation for cell-division: others - a degenerative process: others - a change of form facilitating amoeboid movement (Metchnikoff and Gulland): others - that it is associated with secretory/

secretory activity because of its extensive surface. It is possible that metabolic processes of this nature are some of the most important duties of these cells - the products being perhaps, certain of the anti-bodies. associated with bacterial invasions.

When their function is fulfilled most of the leucocytes degenerate or are taken up by phagocytic cells or wander back into the lymph stream. The various forms of degeneration - plasmolysis and Karyolysis or pyknosis and karyorrhexis are well illustrated in many of my preparations.

2./

2. THE FIBROBLAST:

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(a). Relation to pre-existing connective tissue cells.

There is no doubt that the large proportion of fibroblasts arise by the mitotic division of pre-existing connective tissue cells. The time of the first appearance of mitosis has been put down by various authors as twenty hours (Maximow, Helly<sup>48</sup>). I have never found mitosis in any of my specimens before twenty-one hours: and the height of this activity is reached in about two to three days. It is probable that a stimulus, just sufficient to rouse active proliferation without much primary degeneration would result in an earlier proliferation. The young fibroblasts are capable of migration and are found early in the fibrin strip, but this power is not so developed as in the leucocytes and the mononucleated cells.

(b). Relation to endothelial cells (Page 91.)(c). Relation to mononucleated cells.

Maximow and Ziegler hold that the mononuclear cells are all developmental forms of a mesenchymal/

mesenchymal cell-group which is early differentiated from the tissue forming cells but that in the tissue after inflammation a few may settle as forms indistinguishable from fibroblasts but do not form fibrous tissue. Borst<sup>4</sup>\*, Lubarsch<sup>69</sup>\*, and Adami<sup>1</sup>\*, think connective tissue cells may arise from these mononucleated cells derived from the tissue wandering cells. Adami also adds that mononucleated cells derived from endothelial cells may form connective tissue.

(d). Formation of Fibrils.

The development of the fibrillar inter-cellular substance is of great importance in the process of wound-healing and its mode of formation has been the subject of much controversy: Modern theories resolve themselves into three main groups:-

- (1). That the connective tissue cells secrete a homogeneous substance, which is subsequently rendered fibrillar by the action of mechanical tension (Virchow, Kölliker, Ranvier).
- (2). That the surface layers of the protoplasm become homogeneous and the fibrils originate in these. (Ziegler. Maximow).
- (3)./

- (3). That there is an unravelling of the cell-processes into fibrils together with a transformation of the peripheral layers of the protoplasm into fibrils.

(Schwann, Flemming, Marchand).

Leo Loeb has observed that a transformation into fibrils can be produced by traction upon the protoplasm of cells - fine fibrils passing through the protoplasm of several cells. The same is true of the development of threads of fibrin. It is, therefore, difficult to distinguish between the intra- and extra-cellular processes. As the intercellular substance is derived from the cells it forms a living tissue together with the cells, and the same process may go on in this as in the cells. The fibrils once formed may increase without a direct further participation of the cells. The increase of the fibres, however, is usually held to take place by a direct continuous cleaving off from the sides of the cell-body. In a fully developed condition the tissue is formed of broad bundles - collagen bundles - in which we can no longer recognise fibrils. Between these lie the long, flattened cell-bodies, which appear spindle-shaped on section.

I have found it very difficult with either v. Gieson or Mallory's stain to convince myself of the first appearance of fibrils. The cell protoplasm stains often diffusely with the fuchsin or the anilin blue though I have used these in very various proportions. In some sections such as Slide 144 and Slide 146 ( 5 days) distinct fibrils are found in the cell-protoplasm both on longitudinal and on cross-section of the cells. The processes of the cells also break-up into a sheaf of fibrils. In other specimens (Slides 146, 162 ( 6 days) the border of the cell is deeply stained with the fuchsin. The same appearance is seen in cells which are definitely forming the single walls of blood-vessels (Slides <sup>148</sup> 151-163.) This was so frequent as to lead to the impression that the outer layer of the endothelial cells was formed of a fibrillar substance, or that the endothelial cells rested on a basement membrane (Slide 151 ) At other times (Slide 162 ) there was a very distinct separation between elongated endothelial cells and a single fibril parallel to the outer wall. When the fibroblasts formed a dense plexus of cells (Slide 154 ) the fibrils were found interweaving in the greatest confusion. In the sub-epidermal layers both/



both the endothelial cells of the capillary network and the connective tissue cells were connected by fine processes to the basal cell-processes, forming true cell-bridges between the cells of the epidermis and of the corium. Where the processes of the basal cells were not so marked, there was a very definite intensely stained border to the cells, again giving the impression of a basement membrane. In Slide 4 (I.H.v.G.) the connective tissue fibrils seem to penetrate into the Epidermis.

### 3. THE ENDOTHELIAL CELL.

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- (a). Relation to blood-vessel formation (page 498)
- (b). Relation to connective tissue formation.

For a long time it was assumed that endothelial cells could form connective tissue cells and Baumgarten has found in this circumstance the only deviation from the law that inflammatory proliferations furnish cells of the same kind.

RANVIER (1891) showed that in peritonitis the serosa cells became ramified and fibrillar. Marchand, Roloff, Graser, in studying the healing-in of foreign bodies inserted into the peritoneal cavity, all assumed the transformation of the endothelium into connective tissue.

BAUMGARTEN<sup>"</sup> in 1904 repeated the experiments that had first led him to the conclusion that the blood-vessel endothelium could form true connective tissue. He double-ligatured the carotid in the rabbit in such a manner as not to injure the endothelium of the internal elastic lamina. In the carotid of the rabbit Baumgarten could find no sub-endothelial connective tissue and therefore he excluded this/

this source for the formation of the new tissue. As there were no traces of reaction in the media, and by serial sections he excluded the possibility of granulation tissue extending from the media Baumgarten concluded that the connective tissue growth which arose on the intima was of endothelial origin.

LUBARSCH \* with Baumgarten's views and HEYDE <sup>57</sup> \* (1904) has confirmed his results in every detail.

MERKEL <sup>85</sup> \* and MUSCATELLO <sup>97</sup> \* repeated Baumgarten's experiments and came to an opposite conclusion but as they destroyed the endothelium and ruptured the intima there is a fallacy in their results.

v.BRUNN <sup>16</sup> \* and MONCKEBERG <sup>74</sup> \* have pointed out how unsafe is any inference from omental or peritoneal inflammations and state that it is the underlying connective tissue cells which become star-shaped and fibrillar.

#### (c). Relation to epithelial formation

KOLOSSOW \* has traced intercellular bridges between the endothelial cells.

v.BRUNN <sup>16</sup> \* strongly supports the epithelial origin/

origin of the serosa endothelium on the grounds of the presence of a delicate ciliated border and the fact that in fibrin the endothelial cells may form gland-like spaces. He points out the characteristic of the endothelium to cover free surfaces.

SHULTZE has derived the vessel endothelium and serosa endothelium both from the mesenchyme but according to Hertwig - quoted by Krompecher\*<sup>64</sup> - the serosa cells are true epithelium, but the vessel endothelium is of mesenchymal origin.

The possibility of the proliferated endothelium forming connective tissue cells has been frequently alluded to in the description of my preparations. Plates 27 and 28 show the reaction in the sub-endothelial layers in wounds of six and nine days duration. Tube-like formations, which had evidently arisen from the cutting off of peritoneal indentations in sewing up the wounds, were several times found in my preparations. (Slide <sup>260</sup>/<sub>26</sub>, <sup>278</sup>/<sub>27</sub>). These are lined by a layer of almost cubical cells and illustrate how easily gland-like formations may arise.

I have nowhere found undoubted evidence of the lining of tissue spaces by endothelium. Most text-books say that the so-called tissue spaces are in immediate connection with the lymph-channels, and/

and that here and there an endothelial cell lines the space.

MACCALLUM<sup>72</sup> (1902) has showed that the lymph-vessels form a completely closed system and show no gaps or communications with the tissue spaces.

This does not exclude the possibility that flat cells line the fine spaces of the connective tissue. In the spaces of old scar tissue and around chronic abscesses, the elongated, sessile, mononucleated cells frequently give the appearance of forming an endothelial lining (Slides 50:5:2:224:325<sup>325</sup> 326<sup>326</sup>).

#### 4. THE MONONUCLEATED CELLS.

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Origin: The origin of the mononucleated cells found on the inflamed area is perhaps the most important question which meets us in the study of this subject.

- (1). The discovery of karyokinesis showed that an active proliferation of connective tissue cells took place, and that the progeny of this cell-multiplication consisted of small and large mononucleated cells which were capable of amoeboid movement.
- (2). <sup>141</sup>WOLFF\*, <sup>56</sup>JOLLY\*, and <sup>52</sup>HIRSCHFELD\*. have established the possibility of lymphocyte emigration.
- (3). In the normal subcutaneous tissue are many round wandering cells.
- (4). MARCHAND\* has pointed out the presence of adventitial cells especially around the vessels in the omentum. Under any irritation/



irritation these could rapidly proliferate and produce elements with the characters of lymphocytes and mononucleated cells. These are the Leucocytoid adventitial cells of Marchand.

- (5). RIBBERT\*<sup>108</sup> has drawn attention to the presence of lymphoid nodules intercalated here and there in the perivascular lymph-channels of certain organs and even in the subcutaneous tissues. At the onset of inflammation these rapidly proliferate and form the great mass of the round cells. Beattie has found similar lymphoid sheaths round the vessels of the omentum in the guinea pig and rabbit. Lymphoid cells may also reach the inflamed area by the lymph stream.

- (6). BEATTIE,<sup>12.</sup> HELLY,<sup>48</sup> and others look upon the endothelium, especially in serous inflammations, as an important source of the mononucleated cells. MALLORY\* believes that in inflammation the endothelial cells of the blood vessels and lymphatics/

lymphatics proliferate and pass out into the tissues. ADAMI\* has gone further in this respect and states that the large hyaline blood cells are themselves derived from the endothelium of the vessel.

Under these six considerations I have suggested the possible sources of the mononucleated cells.

1. The pre-existing tissue cells.
2. The emigrated lymphocytes and mononucleated cells.
3. The original round "wandering-cells" of the tissue.
4. The adventitial cells ('Leucocytoid')
5. The perivascular lymphoid sheaths and the lymph stream.
6. The endothelium of the serosa, of blood and lymph-vessels.

By taking up the origin of the mononucleated cells at different periods. I think the question is greatly simplified, for in the early hours of inflammation, several of the possible sources named are excluded. Maximow has taken as the first stage in his investigation nineteen hours because by that time there is no possibility of the proliferation of any/

any of the fixed tissue cells. In the late stages of wound-healing and in many chronic conditions we find definite perivascular infiltrations of round cells - the origin of these cells must also be considered separately. I have taken, therefore, three periods;

- (1). The early hours.
- (2). After the mitotic division of cells has commenced.
- (3). The so-called "small-celled" infiltrations.

I. During the early hours we find in the inflamed area especially near the vessels, very many mono-nucleated cells. The staining of the smaller forms shows them to be identical to the blood lymphocytes. In the larger forms in eighteen hours (Plate *b(6)*) there is already an opening up of the reticulum both of the nucleus and cell-body. The staining is very varied and depends on many factors, but a careful comparison of preparations stained with H.E., E.M.B. M.G.P., with Benda, and especially with I.H., and P.M.B., leaves one with the conviction that a progressive development of the smaller to the larger forms has taken place. Further, in the vessels (Plate *VI b*) are found small and large cells identical to these cells, and in Zenker-fixed preparations instances/

instances of undoubted emigration of these cells can be found (Plate V. fig. b ). The possibility that emigrated lymphocytes and mononucleated cells account for many of the cells in the tissues must therefore, be granted. There can be none derived from proliferation of pre-existing tissue cells, endothelial cells, nor adventitial cells. The perivascular lymphoid sheaths are acknowledged to be very undeveloped in subcutaneous tissue and there remain only the round wandering cells of the tissues, and cells brought by the lymph stream. These certainly, in my opinion add a small number to the mononucleated cells present. All the smaller forms of these cells present morphological characteristics very similar to one another. As they develop the nucleus stains more lightly and the chromatin nodes are more distinct. The weak point in the argument of those who attribute so large a share to the emigrated lymphocytes is the fact that undoubted pictures of emigration are so few. In the great majority of the cells one can recognise irregular projections at the periphery - this goes to prove their amoeboid character.

<sup>122.</sup>  
 SCHWARZ\* indicates how many circumstances must combine to make possible a sure demonstration of/

of cells in this condition.

KIENER and DUCLERT<sup>62</sup>\*, BORST<sup>14</sup>\*, MARCHAND\*, and RIBBERT<sup>108</sup>\* derive the cells from the amitotic division respectively of the connective tissue cells, round "wandering-cells", the leucocytoid adventitial cells and the perivascular lymphoid cells.

Maximow, however, will not admit of any amitosis.

II. In this stage the circumstances are very altered. The different groups of cells just named may all have proliferated and formed a countless mass of cells which cannot be recognised from one another.

BEATTIE\* in experimentally produced peritoneal inflammation in guinea-pigs derives the mononucleated cells from endothelial cells of the serosa, blood and lymph vessel endothelium, hyaline cells of the blood, and cells of the perivascular lymphoid tissue. In taking the peritoneal cavity for the field of his observations Beattie chose an area where the response to infection is - fortunately for patient and for surgeon - admittedly unique. Nature has considerable uniformity in her methods, yet I would venture/

venture to say that inferences drawn regarding the origin and function of mononucleated cells in the peritoneal exudate must be applied with reserve to inflammatory processes elsewhere. The possible source of origin of the cells is so great, and the response so rapid, that the endothelial cells of the serosa should be looked upon rather amongst those cells whose role is the defence of the organism against infection than that of fixed tissue cells whose function is more that of repair. Immense numbers of lymphocytes and true plasma cells are found both in the omentum and in the intestinal submucosa, which render a comparison with the cell-forms in the subcutaneous tissues difficult.

Most recent workers agree with Beattie in deriving the mononucleated cells mainly from the lymphoid tissue, the emigrated blood cells, and the endothelium. The positions taken by Marchand and Ribbert have been criticised on the grounds that the numbers of adventitial or of perivascular lymphoid cells are quite insufficient to account for the large numbers of cells present, and K. Ziegler<sup>145</sup> has pointed out that the vasa vasorum of the larger vessels are engorged, and that by emigration from these the adventitial tissue may become infiltrated by small round cells/



cells (Plate 36. gives a beautiful illustration of a perivascular lymphoid nodule (Ribbert) in the omentum which had become adherent to the wound in the abdominal wall).

In describing my preparations I have pointed out the probability of the endothelial proliferations of blood-vessels and lymphatics sharing with the emigrated blood cells in forming the great mass of the mononucleated cells at this period (Plates 21-23 ). These are, in my opinion, the main sources of mononucleated cells in inflamed subcutaneous tissue. Lymphoid cells from the neighbouring lymphatics add to the number, but most of the mononucleated cells are forms larger than the small lymphocyte of the blood and the lymphoid cell. As emigration still continues - though to a less extent than in the early hours - some of these smaller forms must be newly-emigrated lymphocytes, leaving but few cells to be derived from the lymphatics. After a time it is impossible to distinguish the cells from these three sources.

\*40.  
GULLAND derives mononucleated cells in inflammation from the blood and from all lymphocytes within a reasonable radius, and thinks that there is no necessity to call on the endothelial cell.

### III. So-called "small-celled" infiltrations:

In the later stages of the healing process, and in chronic inflammatory conditions are found numerous perivascular infiltrations of small cells, many of which cannot be distinguished from lymphocytes. The question of the origin of these is quite distinct from that of the mononucleated cells in earlier periods, but the possible sources are the same.

MARCHAND's view of the increase of the adventitial cells cannot be excluded at this stage: for the cells are 'leucocytoid', are around vessels, and mitosis can be found.

MAXIMOW still holds to his view of the origin from emigrated blood-cells, and especially in suppurative inflammation gives pictures of emigrating lymphocytes.

MAGCALLUM<sup>71</sup> refers to a case of acute meningitis, where the meninges were infiltrated with lymphocytes. This process occurring in a tissue ordinarily devoid of lymphoid tissue could be explained only by an emigration from the blood-vessels in response to a chemiotactic influence.

RIBBERT states, in support of his view, that the accumulations are around the larger vessels from which there could be no emigration; but

K./

<sup>145</sup>  
K.ZIEGLER observes that the vasa vasorum are engorged and the emigration could arise from these vessels.

<sup>69</sup>  
LUBARSCH also points out that the infiltrations contain numerous acidophile cells which must have emigrated from the blood-vessels.

The endothelial cells of the perivascular lymphatics are also held, by proliferation, to add to the source of these cells. The presence of an endothelial lining to these spaces is denied by many. I have frequently found evidence of the proliferation of the lining of spaces around the larger vessels, and of the loosening of these cells into the lumen (Slide 300). The presence of larger cells than the lymphocyte in these infiltrations may thus be partly accounted for; for the proliferated endothelial cell is not a small lymphocyte-like cell.

PAPPENHEIM<sup>\*100-101</sup> is perhaps the greatest combatant on this field. His arguments are so bewildering that his position is very difficult to place. As far as I have been able to follow him, he admits migration of the lymphocytes, but denies pervasal emigration except in general lymphocytosis. The perivascular infiltrations arise as a proliferation of perivascular histiogenous lymphocytes. This is analogous to the formation of Malpighian follicles round/

round the arteries in the spleen.

My observations of these infiltrations are limited to the late stages of slowly-healing wounds and abscesses. They tend to confirm the views of those who derive these cells from the lymphatics. In inflammation, especially where the irritant has been acting for a long time, the cells of lymphoid tissue proliferate and may soon form lymphoid nodules in any area where the first cells brought by the perivascular lymphatics have collected. Frequently the lymph-channels near these vessels and the tissue spaces can be seen filled with rows of such cells. (Slides <sup>264</sup><sub>270</sub>, <sup>271</sup><sub>272</sub> ).

Function of the Mononucleated Cells. Again, I distinguish the mononucleated cells during the inflammatory process from those in the small-celled infiltrations. All observers are agreed that the chief function of the former cells is the clearing away of the waste products on the inflamed area, and the preparation of the region for the fibroblasts. That observers differ so greatly as to their origin and their destiny makes the unity of conception of their function all the more remarkable.

Muir\*<sup>96</sup> has pointed out that the spherical form/

form and phagocytic function represent very primitive properties in the process of evolution, and therefore cells of different classes may revert to the former state existing before differentiation and specialisation.

These cells have both an intracellular and an extracellular activity. Metchnikoff looked upon *unicellular glands and regarded the vacuolation as* them as a result of an abundant secretion of digestive fluid. Beattie has suggested that when the cell-protoplasm stains darkly there is possibly an accumulation of some substance - probably a secretion of the cell. When the cell begins to function actively, this secretion is discharged either as a nutritive agent or as some special ferment or possibly antitoxic body, and the cell becomes clearer and vacuolated. It is certain that as pus phagocytes, these cells not only digest in their cell-bodies the products of disintegration which they have absorbed, but are able to dissolve the mass which surrounds them. This is specially noticeable in the latest stages, where they occupy special cavities in the thickened pus.

<sup>135</sup> WADE, in considering the aetiology of Infective Sarcoma of the dog, thought that the causal factor in the growth of the tumour might be the absence of the mononucleated cell. When tumour portions/

portions were inoculated into animals the response on the part of the host consisted in the production of lymphoid cells in excess. As the tumour growth is arrested these mononucleated cells replace the tumour cells, and Wade holds that this arrest in growth is probably due to the creation of some substance carried by the mononucleated cell.

The function of the mononucleated cells in the small-celled infiltrations will be considered under Plasma cells.

Fate: they may

- (1) Undergo disintegration locally;
- (2) A few may return to the circulation;
- (3) May form giant cells by fusion or by direct division of the nuclei;
- (4) May be factors in the formation of new tissue.

(page 86.).

(5)/

## (5). PLASMA CELLS.

<sup>77</sup>  
 Marschalko's work has settled definitely the conception of the plasma cell. In most of my preparations of slowly-healing wounds and in the abscess-membrane typical plasma-cells were found in large numbers. The structure of those cells is in these illustrations very beautifully brought out: the characteristic 'hof' with the specially differentiated centrosome apparatus, the eccentric position of the nucleus, with radiating lines to the central chromatin mass forming the typical radkern nucleus. As a result of very careful examinations of many preparations stained with P.M.B., M.G.P., and I.H., I agree with those who look upon the plasma cell as a morphological unit.

<sup>131</sup>  
 UNNA<sup>131</sup> in 1891 first described these characteristic cells in lupus. Since that time they have been recognised to form a constituent element of many "small-celled" infiltrations both of the infective granulomata and many chronic inflammatory processes. Unna describes them as granular cells - granular, not in the sense of Altmann and Ehrlich, but granular as opposed to homogeneous. Marschalko, who called in question/



question nearly all of Unna's statements in regard to this cell, accepted this feature. It was left to SCHRIDDE<sup>119-120</sup>\* by means of formol-Müller-osmic acid fixation and anilin-fuchsin staining to demonstrate that these cells had true granules, and that the characteristic 'hof' is only a gathering together of the unstained granules.

Schridde considers with Ehrlich that the granules are metabolic products of the cell, and not stationary structural elements. In regard to their origin he lays special stress on the size, colour, and arrangement of the granules, and finds that the small lymphocytes in the perivascular infiltrations have similar granules. Schridde traces all transitions between the small lymphocytes with few granules in the centres of these infiltrations and the true plasma cells which develop<sup>only</sup> on their periphery, and naturally derives the plasma-cell from the lymphocyte. Schridde has found that the lymph-gland cells have similar brick-red granules, but the lymphocytes of the blood are distinguished by their smaller size and the brownish-red granules. Schridde derives the plasma-cells not directly from the lymph-glands, but from the small groups of lymphocytes proved by Saxer and by Gebert<sup>35</sup>\* to invest the vessels in the embryo.

I look upon the "small-celled" infiltrations as essentially consisting of lymphocytes in process of development to plasma-cells. The identity of their nucleus is very marked, and every stage of transition in size and form can be found in one of these cell-infiltrations. These lymphocyte cells appear to come by the perivascular lymphatics. In favour of this is the frequent appearance of rows of cells in the lymphatic spaces and the small lymphocyte accumulations in many tumours can be explained by the obstruction of the lymph-vessels to the part.

Function. Lymphocytes are specially called out in chronic processes, where the virus is not so intense in action, but yet is very persistent. Their function may be that of uni-cellular glands. The development of the granules, as the cells increase to become plasma cells may be due to the special need of some substance, the nature of which can as yet be problematic. In the abscesses undergoing slow absorption I found a very great penetration of the plasma cells amongst the vacuolated cells (Slides 323-326, 22-24), and it may be that they have the task of making non-injurious decomposition products, or, as Justi has suggested, carrying some substance to/

to the proliferating formative cells for their nourishment, or carrying out of the tissues a substance, by their removal in the lymph stream.

WHITFIELD<sup>137</sup> believes these cells to be closely associated with the question of immunity - possibly local immunity, and MALLORY<sup>75</sup> has suggested that in typhoid the plasma cells may produce the antitoxin. I have never seen these cells phagocytic.

Fate. Very frequent degeneration forms are seen in the tissue spaces and lymphatics, very suggestive of their having fulfilled some function. (Slide 54).

## M U S C L E   T I S S U E.

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No changes in relation to the healing of wounds have been more difficult to interpret than those in muscle. This arises from various factors chief among which are the close association between the degenerative and regenerative processes and the difficulty of distinguishing between the cell elements descended from the proliferated sarcolemma nuclei, the endomysium, and the endothelium. After the borders between muscle fibres have disappeared we find side by side large muscle-cells, fibroblasts, endothelial cells, polymorphonuclear leucocytes, and large mononucleated cells. The whole forms a cellular tissue in which one can no longer recognise the descent of individual cells. Later, elements appear which for many years have been taken by some investigators as signs of regeneration and by others of degeneration.

## H I S T O R I C A L.

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At one time regeneration of muscle tissue was regarded as impossible but cases occurred where the production of new muscle fibres must be postulated/

postulated e.g. Askanazy described such in tumours and Weber\* rarely found connective tissue scars round old fractures.

Zenker (1864) showed that in typhoid fever there was a regeneration of muscle fibres. He derived them from spindle-shaped cells from the sarcolemma, which by nuclear division gave rise to ribbon-like formations which finally became muscle fibres.

Kölliker, Waldeyer, Weber (1868) described muscle-cell-tubes filled with cells. Weber derived these from muscle cells and Waldeyer, from the cells of the endomysium. Certain of these cells became free and spindle-shaped and by nuclear division form the ribbon-like elements which represent the transition to muscle fibres. Later workers showed that these free muscle cells could migrate and thus was explained the appearance of new fibres where previously no muscle elements had existed.

Kraske\* derived the muscle cells or muscle-cell-tubes from proliferated sarcolemma nuclei and showed that the cells increased in length by direct nuclear division and at the end of the third week became transversely striated. Kraske's work has gained many adherents to the view of the origin of muscle/

muscle fibres from muscle cells.

Budge and Weissmann\* found an increase of muscle fibres by longitudinal cleaving of the old fibres.

Neumann\* (1868) described the development of new muscle fibres by "budding" from the preserved muscle fragments and stated that this was the sole mode of origin of new muscle fibres. Zenker and Volkmann on the other hand, maintained that the muscle-cells are the most active agents in regeneration.

Barfurth (1891) showed that these two processes are not mutually exclusive and Volkmann\* (1893) that Neumann's "buds" and the "muscle-cells" are both of purely sarcoplasmic origin.

From this time the active agents in regeneration were recognised to be muscle-cells or buds. In the one case the sarcoplasm is individualised into distinct cells: in the other it remains in plasmodial indivision. The bud is simply a muscle-cell that has remained in contact with the contractile substance of the preserved muscle fibre.

## HISTOLOGICAL EXAMINATION.

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The immediate consequence of the incision was the contraction of the muscle and the filling of the gap with blood. The cut ends of the muscle fibres showed even in six hours as structureless, brightly-staining, (H.E.) clods surrounded by fibrin and leucocytes. Owing to the irregular approximation of the layers of the abdominal wall these muscle remains were found in varied relation to the wound edges. I shall describe the appearances in a wound where the surfaces, in part at least, were kept in more or less apposition.

Plate 37 shows the different zones of a muscle-wound:-

(a) The central fibrin strip, at the top of the figure containing dense masses of leucocytes and chromatin particles and surrounding at its borders debris of muscle fibres. These are of irregular contour, without nucleus, and without any structure. Many of them are penetrated by leucocytes and are on the point of dissolution by histolysis. (slides 37 and 238).

(b)/



(b) Passing outwards from this necrotic zone we come to muscle fibres which have almost the same bright staining but have retained their nuclei. Around these and within them are a few leucocytes and red blood cells. In some parts the muscle fibres are completely replaced by leucocytes and these in turn show the same bright staining as if they had, by phagocytosis, absorbed the muscle substance as well as by their extracellular action caused its dissolution. (Plate 7, fig.a.).

(c) Farther out still are found groups of muscle fibres with a granulation tissue separating them. The muscle fibres show on transverse section groups of round cells which are still enclosed in the sarcolemma sheath or the endomysium. These cells show an intense phagocytic activity to the muscle substance. (Plate 7, fig.b.) In the granulation tissue surrounding these muscle fibres are numerous similar cells containing the remains of muscle substance. Here and there are found fibres replaced by cells which give the impression of having resulted entirely from the proliferation of the sarcolemma nuclei and of having surrounded themselves with sarcoplasm, while at the same time they are intensely phagocytic/

phagocytic to the remaining myoplasm. All stages of transition are seen between the first proliferation of the sarcolemma nuclei, forming a ring round the muscle substance, and the complete absorption of the muscle and its replacement by uninucleated cells. Amongst these individualised cells I have frequently found mitosis (Plate 8, fig.c.) but never while still remaining part of the muscle fibre.

At other parts the cells within these tubes are definitely penetrated elements and consist of leucocytes, mononucleated cells, and red blood cells: while still other fibres show a distinct proliferation of the sarcolemma nuclei with penetration of numerous cells at the same time.

Similar appearances on longitudinal section of the fibres are still more difficult to interpret. Slides 8 and 27 show the appearance of long tubes filled with a mass of cells of very varied origin, and Plate 38 shows longitudinal fibres almost completely replaced by round cells, which stain very deeply with pyronin. (Slide 38). Around the muscle fibres thus breaking up we get a very active proliferation of the cells of the endomysium and of the capillary/

capillary endothelium and soon these "muscle-cell-tubes" - for such they must be called - are surrounded by a meshwork of branching fibroblasts and numerous young vessels. Into this granulation tissue the muscle-cells escape and can no longer be distinguished from the other mononucleated cells.

Nearly all writers have represented the muscle-cells as round elements, but Saltykow by means of a modification of Nissl's staining found that they were branching cells. He concluded that they had arisen by an extension of the original sarcoplasm substance around the proliferated nuclei. In adult normal fibres the sarcoplasm - the undifferentiated protoplasm - is very small in amount but in pathological conditions it increases first around the nucleus and, extending, makes visible the connections between the nuclei - thus forming anastomosing processes of cells. The pyronin stain also brings out beautifully the processes of all cells and slides 8 and 35 show very numerous examples of cross-sections of muscle fibres replaced by an anastomosing network of cells enclosed still in the endomysium sheath. Several of these cells are in mitosis, (Plate 8 fig.c.), and frequently remains of/

of muscle substance may be recognised within them, as well as surrounded by them.

It is in the area farthest removed from the fibrin strip that we find fibres replaced by round or anastomising cells which give the impression of being derived solely from the sarcolemma nuclei.

(d). Instead of the proliferated nuclei becoming individualised the protoplasm may remain undivided or we may have fusion of penetrated mononucleated cells around the muscle substance. In this way are formed multinucleated masses of protoplasm, which have the significance of muscle giant cells. Plate 8 (figs. 4 and 5.) shows two stages in the formation of these and Plates 39 and 40 and numerous slides illustrate all stages in the transition from the earliest proliferation of the sarcolemma nuclei to the formation of multinucleated, usually crescentic-shaped masses around the remains of the myoplasm. This is seen in both longitudinal and cross-section; and according to the direction of the section we get all variations in form and size. (Slide 33). The proliferation of the sarcolemma nuclei is the first effect of an irritant which/

which has not been severe enough to destroy the whole muscle fibre - sarcoplasm as well as myoplasm. These muscle giant-cells show intense phagocytosis to the muscle substance and also to red blood cells and leucocytes. These multinucleated protoplasmic masses may later break up into individual cells.

(e). In a zone still farther removed from the centre necrotic area, we find in some wounds later on that the preserved muscle fibres have multinucleated swollen ends. It is difficult to distinguish between these and the earlier muscle-giant-cells described under (d), especially on cross-section. Many of these earlier forms must be also the end-segments of preserved muscle fibres with proliferated nuclei but in a fortunately-stained section, especially with M.G.P., the end-stumps are recognised as elements of regeneration, <sup>while</sup> the giant cells enclose degenerated muscle substance. These swollen end-stumps with many nuclei must be regarded as the muscle-"buds" of Neumann. (Plate 9, fig.a.). When muscle segments whose nuclei have thus proliferated are cut off from the preserved segment we get true muscle giant-cells. These can be recognised in large numbers in the early scar tissue, (Plate/

(Plate 42) and remain as such for a long time. In my oldest wounds of sixty and seventy days they are still to be found.

(f). There remains still to be described one definite appearance in relation to the muscle changes in wounds. In a few places the muscle fibres become disassociated into fibrils (Slide 178.). This fissuring has in many cases every sign of a degenerative splitting up of the fibre into its constituent fibrils. From other fibres, however, either from the sides or from the ends long, narrow, spindle-shaped portions with elongated nuclei, break off. (Plate 9, fig.b.). In the surrounding tissue all transitions from these to long ribbon-forms with many nuclei can be traced. (Slide 171.).

#### INFERENCES FROM THESE APPEARANCES.

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The interpretation of this histological picture is a very difficult one. The structureless muscle debris may remain for a long time unabsorbed and even in the pus mass (Slide 294.) are long in undergoing complete dissolution. The muscle fibres penetrated/



penetrated by leucocytes and other cells disappear and are replaced by a very cellular granulation tissue. Muscle-giant-cells remain, as we have seen, for a long time in the scar tissue, the centre often being of hyaline structure. In several sections (slide 28) I have been able to trace the breaking up of these giant-cells into round and spindle-shaped elements. These pass into the granulation tissue and resemble the other cell-forms there and probably ultimately disappear.

It is when we come to the significance of the "muscle-cell-tubes" filled with round or anastomosing cells that difficulties begin. In wounds I have seen nothing to indicate that the muscle-cells set free in the granulation tissue become spindle-shaped cells, which by nuclear proliferation and differentiation of protoplasm form transitions to young muscle fibres. Zenker and Volkmann have described this process in typhoid fever where only the myoplasm of the muscle-fibre is injured. Cornil and Ranvier<sup>22</sup> have given the name "Regeneration by Embryonic type" to this form of regeneration, in opposition to that by "Budding" (Neumann). There is evidence that where the muscle fibres/



fibres are not severely injured, regeneration may take place in this way. Plate 43. represents a very slight wound through the superficial muscle (panniculus carnosus) twelve days old. This narrow fissure gives an opportunity to study the muscularisation of a small scar. In the centre of the scar numerous round cells are seen with a broad edge of protoplasm. All stages of transition between these and cells undergoing complete karyolysis and cytolysis are found in specimens stained with V.G., H.E. and M.G.P. These are distinctly the set-free muscle cells which have remained in the scar tissue recognisable as elements descended from muscle but definitely undergoing dissolution. Nearer to the muscle fibres projecting into the scar tissue are spindle-shaped elements which will be referred to later. Therefore in the granulation tissue which takes the place of the destroyed muscle fibres and in its neighbourhood muscle-cells can be recognised if the reaction has not been too great. Where there has been excessive proliferation of all the tissue elements, endomysium and endothelium, the set-free muscle-cells can no longer be recognised and it is unlikely that any of them can become spindle-shaped and/

and form new fibres.

There remains then to be considered only the ends of the preserved muscle fibres, which we have seen have frequently bulbous multinucleated endings. If we consider the wound fissure - in relation to the muscle - as a central zone of necrosis and degeneration, on either side we have a zone of cellular regression where the muscle fibres have been transformed into elements which take no share in muscle regeneration (in a wound). Farther out on either side we have a zone of possible regeneration from the ends of the preserved muscle fibres which project into the intervening scar tissue. To what extent can this intervening area be muscularized and how does it take place?

Plate 44 shows such a muscle wound (21 days). The zones of degeneration and cellular regression have been replaced by a cicatricial tissue. In the zone of possible regeneration muscle fibres with tapering ends or with club-like enlargements radiate into the scar tissue. These muscle-endings have numerous nuclei and show a distinct fibrillation between the nuclei. Many of the fibres are disassociated into fibrils which show a similar/

similar increase of nuclei. By the multiplication of the nuclei these fibre-ends increase in length, and by a differentiation of the sarcoplasm around the nuclei increase in breadth, and thus multinucleated bands or ribbons of protoplasm more or less fibrillated are formed. (Plate 9, fig.a.). According to the plane of the section such ribbon-formations may be found isolated in the tissue. The free extremity is usually a homogeneous multinucleated mass of protoplasm which will later become fibrillated.

Plate 9 (fig.6) illustrates another possible mode of regeneration of muscle fibres. From the ends and sides of the muscle "buds" spindle-shaped portions of nucleated protoplasm are cleft off - these escape into the neighbouring tissue and by nuclear proliferation and increase in sarcoplasm may also form ribbon-like elements, which later become new muscle fibres. Slide 171 shows numerous examples of such spindle-shaped single elements or multinucleated ribbons lying in the spaces of the connective tissue. Several possibilities here present themselves. Are these not dwindling and atrophied old fibres on the point of disappearance or/

or are they new cleft-off fibres which, through the increasing pressure of the fibrous laminae, are becoming atrophied or are they these cleft-off fibres in process of evolution to form new fibres? The staining of these elements, especially with M.G.P. is not that of old fibres on the point of disappearance but has the clear pyronin staining of the undifferentiated muscle substance. As it becomes fibrillated it assumes a purplish tint.

These elements - "buds" and cleft-off fibres are said to undergo a further longitudinal division independent of the position of the nuclei which later come to the periphery. The final stages in their evolution are the transverse striation and formation of a sarcolemma sheath.

If these forms seen in the zone of regeneration are new muscle fibres, to what extent can they muscularize a cicatrix? Cornil and Ranvier state that this cannot take place more than one or two millimetres on either side, so that a cicatrix of more than three or four millimetres will not be perfectly muscularised. Probably the condensation of the fibrous laminae being laid down prevents these young fibres from advancing further.

Proliferation/

Proliferation of nuclei. The rapid proliferation of nuclei seen during the first six to twelve hours points to amitosis. This mode of division is characteristic of highly specialised tissues and there are no signs of mitosis. I have been able to find only one instance of what seems an undoubted mitosis in a sarcolemma nucleus that had not formed an individualised cell (Slide 263.). This proliferation of nuclei results in the formation of the muscle-cells and in these many instances of mitotic division can be found in one preparation and even in one field (Slides 8 and 35). This mitotic division is characteristic of embryonic cells and must be regarded as the general expression of the "eternal law of continuous development" on which Virchow insisted. These muscle cells under favourable conditions would, according to Volkmann go on to form new muscle fibres, and in these near their final evolution the division again becomes amitotic. In the "buds" and cleft-off spindles the growth in length is again by direct division, and long muscle elements are frequently found with closely applied rows of nuclei. (Slides 271, 275, 276)

Time of appearance. It is difficult to lay down dates regarding the time of appearance of these/

these various changes for in no tissue are degenerative changes so easily and rapidly brought about as in muscle. Burkhardt's<sup>17</sup> investigations show that the injection of an extract of bruised muscle-substance acts chemiotactically and can produce aseptic suppuration, so that where blunt knives and other circumstances have caused much damage, the leucocyte reaction is likely to be very great. I have found proliferation of nuclei in most of my wounds of six hours. The muscle-cell-tubes are present very definitely on the third day, frequently on the second day; the early giant-cells, on the fourth day, the later giant-cells in the scar tissue about the twelfth day. Ribbon-like elements as early as the fifth day. Weber, who believed that spindle-shaped cells arisen from the muscle-cells could form ribbon-like elements, traced transverse striation at the end of the third week.

#### CONCLUSIONS RELATING TO MUSCLE CHANGES IN WOUNDS.

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- (1) That degenerative and regenerative processes are very closely associated.
- (2) That early proliferation of nuclei occurs in/



in all parts of the preserved muscle fibre within the area of reaction. This cannot be looked upon always as a regenerative appearance for it accompanies changes which evolve in two very opposite directions.

- (3) That "muscle-cell-tubes" are formed from the second day onwards. Some of these contain only muscle-cells from the proliferation of the sarcolemma nuclei. Others contain muscle-cells and penetrated elements all of which are phagocytic to the myoplasm.
- (4) That muscle-cells set free in the granulation tissue may be recognised for a time there, but that ultimately they disappear or form cells which cannot be distinguished from the surrounding cells and that they take no part in the regeneration.
- (5) That muscle segments no longer in connection with intact muscle substance form multinucleated masses of protoplasm - muscle-giant-cells - which are frequently very numerous in scar tissue.

(6)/



- (6) That regeneration may take place to a slight extent from the ends of preserved muscle fibres by a process of "budding" or of cleaving-off of spindle-shaped nucleated elements. By a further nuclear proliferation and increase and differentiation of the protoplasm ribbon-form elements are produced which are the transition stages to new fibres.
- (7) That these young fibres in the scar tissue to a great extent atrophy with the increasing condensation of the scar tissue.
- (8) That the cellular conception of a muscle fibre in opposition to the fibrillar conception dominates the whole pathology of muscle tissue.

ELASTIC/

## ELASTIC TISSUE.

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### (1) INTRODUCTORY.

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Elastic fibres are found very evenly scattered through the framework of the skin and form a complete mesh-work between the collagen bundles. Some of the fibres are as large as the collagen bundles and are connected by very delicate branching fibrils. Surrounding the vessels, nerves, hair-follicles, and glands this meshwork is composed of extremely fine fibrils. The function of this elastic framework is probably that of an inhibitory apparatus which, in general, equably distributes pressure and traction, and specially helps the involuntary muscles to regulate secretion on the one hand, the circulation of the blood on the other, and in this way to influence the movements of the fluids and the interchange of gases.

In acute inflammatory conditions such as occur in the healing of wounds with considerable reaction there is very marked destruction of the elastic fibres, although these are relatively resistant in comparison to the other tissue elements.

Plate/

Plate X gives a low power view of a wound seven days old in which there is complete absence of elastic fibres in the scar tissue. At the borders, we find the fibres suddenly interrupted, more or less thicker, and staining a little more diffusely than normally. A few curled up fibrils may also be seen arising from these, but these are very scanty. At one part there is a streaming into the scar tissue for a short distance of straight wavy fibrils - this is in connection with a small blood-vessel.

Plate XI. gives a low power view of a wound five months old. The nodule of scar tissue is wedge-shaped with the base at the surface, and is distinctly outlined by elastic fibres on all sides. A broad band of very delicate elastic fibrils stretches in the superficial layer from one edge to the other, a short distance under the epidermis: and on each side radiating elastic fibres penetrate the scar tissue. A wide central zone is, under the low power, entirely devoid of elastic tissue, but under high power, numerous fine granules and wavy fibrils are found in it. The skin papillae are very undeveloped and the beautiful subepidermic network of elastic fibres is absent.

It/

It is only in the superficial layer that the fibrils reach across the scar. Here we see fine fibrils branching from the old deeply-stained fibres at the edge. The appearance is that of the trunk of a leafless poplar tree with numerous branches all running for a considerable distance nearly parallel to the parent stem. From each branch innumerable twigs are given off encircling the cells met with on the way. The longer twigs are often in such close relation to the cell that they form its border, while the finer twigs have, as often, the appearance of branching off from this border as the cell-processes.

In the scar tissue itself we find numerous stained dots scattered in groups, especially in close relation to the vessels; and in other parts wavy, cork-screw-like fibrils often in very close relation to cells. In the denser parts of the scar tissue the fibrils are very scanty. At the borders in the old tissue homogeneous swollen fibres are here and there found but along almost the whole border on each side, and from the deeper parts elastic fibrils stream into the scar tissue in the same manner though with a more wavy course than in the superficial/

superficial layer.

A consideration of these two preparations raises many points of interest. How have the elastic fibres disappeared in the wound of seven days, how have the new-formed fibres arisen in the wound of five months, and what is the significance of the regeneration?

## (2). DEGENERATION.

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Degenerative changes leading to disappearance of the elastic elements in wounds are difficult to trace owing to the great disorder in the relative position of the parts. In the interstices of the wound edges we find that the elastic fibres here and there have not the clear outline of the normal fibre, also that fragments of these homogeneous swollen fibres are often scattered in the scar tissue in clumps. In scar tissue where there is no definite sign of regeneration delicate, faintly-stained fibrils may be traced. These may be either the disappearing fibrils losing their power of specific staining and in process of gradual dissolution (Miller<sup>92</sup>) or newly-arisen fibrils which have not yet attained/

attained this quality (Jores<sup>57</sup>\*). The presence of these fibrils indicates that a breaking-up of a fibre into its constituent fibrils is part of the process of degeneration, that these lose their staining reaction and finally disappear.

In the abscesses to be discussed later there was very rapid destruction of the elastic tissue, both in connection with the walls of the vessels and in the tissue. The earliest disappearance was at the zone of inflammatory reaction; farther away the elastic filaments were often long, straight threads as if stretched excessively (Slide 206) in consequence of the swelling. Both in the zone of the granulation tissue and in the fibrous tissue capsule up to fifty days I found an entire absence of elastic fibres.

Du Mesnil de Rochemont considered that degeneration of elastic fibres was due to a chemical action of the inflammatory products. In tuberculous inflammation he believed the disappearance was the result of free toxins - the products of the tubercle bacillus.

Melnikow-Raswedenkow<sup>94</sup>\*, Miller<sup>92</sup>\*, Jores<sup>58</sup>\* explain that the factor in the production of the degeneration/



degeneration is the inflammatory cell-infiltration and point out the significance of the cell-infiltration in this relation in Tubercle.

Obermüller found the cause in the purely mechanical action of pressure. Katsurada, however, crushed the skin of animals and found that direct severance in continuity of elastic fibres had not occurred unless the injury had been severe enough to cause similar changes in other tissue elements. As a result, however, of the inflammatory reaction set up by the injury the elastic fibres disappeared.

### (3) R E G E N E R A T I O N

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#### (a) STAINING.

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A regeneration of elastic fibres in scar tissue has been admitted ever since the introduction of elective staining methods. The earlier investigators concluded that any elastic fibres found in scar tissue were the remains of old fibres (Gutten-tag<sup>41</sup>\*). I have used Weigert's staining method: differentiating for a short time in acid alcohol and for thirty minutes to one hour in ordinary absolute/



absolute alcohol. In many cases I have used as a control a differentiation with picric acid as recommended by Mall<sup>\*74</sup> and Miller<sup>\*92</sup>. Miller has pointed out that by means of picric acid faintly stained fibres, which have not taken the resorcin-fuchsin stain, may be followed up. Jores states that the "vorstufe" of elastic fibres are delicate refractile fibrils which do not take on the characteristic stain.

Fuss<sup>\*33</sup> considers that staining is only an incomplete medium for representing the first beginnings of elastic fibres and Jacobstal, Schiffmann<sup>114</sup>, and Goldmann<sup>\*36, 37</sup> agree with Jores that in their first arrangement the finest fibres do not stain differentially. Teuffel<sup>125</sup> and Fischer<sup>\*30, 31</sup> have pointed out the significance of the staining of other substances with elective stains. Mall<sup>\*74</sup> has treated frozen sections of the skin of embryos in boiling dilute caustic potash and has found that only a network - the elastic fibres - remain. At the same stage of development the skin in young embryos showed no elastic network by Weigert's method.

Fischer<sup>\*30, 31</sup> has very thoroughly worked out the chemistry of Weigert's stain. He has showed that if the fuchsin is left out of the mixture we get/

get a mordant in the ferri-resorcin, after the use of which a group of chemically related pigments, such as safranin, vesuvin, and fuchsin, will stain the elastic fibres. Neither ferric chloride alone nor resorcin can thus make the elastic fibres accessible to the pigment. Fischer has gone further and proved that other substances than elastic tissue stain thus, but that a careful differentiation in alcohol removes the stain from these structures. He believes that the retention of the stain by the elastic fibres points to the definite formation of a new pigment with the fuchsin.

#### (b) HISTORICAL.

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Origin of the new fibres: On the mode of origin of the new fibres three theories have been advanced:-

- (1) That the young fibres are offshoots from old ones.
- (2) That they arise "de novo" in the scar tissue from cells.
- (3) That they arise "de novo" in the scar tissue from intercellular substance.

I. Origin from pre-existing fibres.

Goldmann<sup>36 37</sup> (1894 and 1901) is the chief upholder of the view that the new fibres arise in relation to old ones. His investigations in regard to Keloid and scar tissue convince him that the sources of origin are the adventitial sheaths of vessels and hair follicles adjoining the nodules. In hypertrophic scars he found elastic tissue in degenerated clumps from which there was no regeneration. In transplanted skin grafts Goldmann came to the same conclusion that the peripheral elastic tissue was the source of the new elastic fibres. Enderlen agrees with Goldmann but admits that new tissues arise from the old fibres in the grafts.

Miller<sup>92</sup>\* studying the changes in the elastic tissue in the lung in simple, tuberculous, and syphilitic inflammatory processes observed the origin of new fibres from pre-existing ones both of the pleura and of the vessel walls. Miller believes that new fibres also arise in the intercellular substance probably in relation to cells.

The origin of fibres arising in loco takes us/

us back to the embryonal method of formation. It is recognised that development under pathological conditions is frequently the repetition of a certain period of development and numerous investigators have approached the subject from this standpoint. There are many analogies between the connective tissue fibril development and that of elastic fibrils. The same two possibilities meet us:- Do the fibrils arise -

- i. Cellularly - in relation to the cell protoplasm (Gardner, Touffel<sup>125</sup>, Hansen, Minervini<sup>93</sup>, Nakai<sup>98</sup>, Jores<sup>57-59</sup>) or
- ii. Intercellularly - as a result of some change in the ground substance? (Kolliker, Ranvier, Katsurada, Linser, Melnikow-Raswedenkow<sup>74</sup>, Fuss<sup>33-34</sup>, Guyot<sup>43</sup>, Schiffmann<sup>114</sup>).

Again it is undecided by workers on both sides whether:-

- i. Elastic granules deposit themselves and through subsequent blending form fibres (Loisel, Ranvier, Gardner, Jores) or whether
- ii. the transformation is carried out regularly throughout the whole extent of the fibre without/

without any granule stage. (Kolliker, Minervini, Nakai, Schiffmann).

## II. Cellular origin.

Loisel described special cells - elastoblasts - in the ligamentum nuchae of the embryo of different animals.

Gardner\* in the investigation of the foetal membranes found extraordinarily fine granules in the protoplasm of mesenchymic cells. These granules arrange themselves in rows and come to lie outside the cell where they blend to form thin threads. Growth takes place through a fusion of these thin fibres.

<sup>78</sup>Nakai\* in embryos found that the first elastic fibres are the direct continuations of the processes of mesenchyme cells. The first fibres develop in the walls of the Aorta and Pulmonary artery at a time when no finished connective tissue fibrils are present.

Investigations in embryonal tissue speak very decidedly in favour of the direct descent from the cell but the same appearances, though not so frequently, are found in the study of regeneration in all varieties of tissue.

Minervini/

Minervini<sup>93</sup>\* in the scar tissue of wounds, believed that the processes of spindle-cells gave origin to the elastic fibres.

Taddei<sup>148</sup> saw in scars the direct transformation of the protoplasm of the cell into thin elastic fibres which later isolated themselves from the cell.

Jores<sup>59</sup>\* (1907) both in the embryo of fowls and in scar tissue found the first elastic elements as delicate granules which show the same arrangement and form as the protoplasm processes of the cell. By means of a modification of Pappenheim's pyronin stain together with Weigert's stain, Jores found thin blue granules and fibrils appear as continuations of the deeply pyronin-stained cell protoplasm. These fibrils rapidly get beyond the first stage and become independent of the cell and Jores believes that the upholders of the intercellular theory interpret this as the first stage of the elastic fibril. Jores attributes to the cells of the intima a special capacity for forming elastic tissue.

Heubner believed the origin of the internal elastic lamina was from the endothelial cells.

Several/



Several of the above writers admit that a development from the intercellular ground substance may also be possible.

### III. Intercellular origin.

The reasons given by the opponents of the cellular theory are mainly of a negative kind and culminate in this that no connection between elastic fibres to cells can be traced.

Ranvier and Reinke believe that the first appearance is that of granules in the intercellular substance. Schäfer regards the proof for the origin of elastic tissue insufficient, but thinks that the extra-cellular deposition of granules is very probable.

Kölliker holds that the fibre arises as a whole, though in very great fineness, by a specific transformation of the ground substance.

Ziegler and Miller believe in the differentiation of the fibrillar ground substance; Schiffmann, that the connective-tissue fibres transform themselves "in continuo" into elastic fibres; Fuss\*, that in the connective tissue fibril an axial string of elastin is formed, which/



which finally takes possession of the whole circumference of the fibril; Guyot, that there is a primitive differentiation of an amorphous intermediate substance.

### (c). SIGNIFICANCE.

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Many writers ascribe to the elastic tissue a teleological significance - relating it, as Schiffmann has done, to the mechanical requisition of the part. If the view of the function of elastic fibres in the skin stated in the introductory paragraph of this chapter is correct, then it is impossible not to see in the regeneration an endeavour to restore the disturbed equilibrium of the tissue.

Many examples are given by Melnikow-Raswedenkow and other writers of the relation of the development of elastic tissue to the function of the organ. Livini<sup>67</sup>\* has showed the relative proportion which exists between the amount in glands and the nature of their secretion. Physiological or pathological atrophy of an organ, leading to an impeding of the circulation, results in an increased elastic tissue development - to facilitate the function of the/

the remaining gland elements. The amount of increase is in inverse ratio to the atrophy of the specific tissue elements. In arteries an increase of elastic tissue is in proportion to their decrease in contractility. Again, therefore, an effort to restore disturbed equilibrium. Parts which require more "elasticity" have, therefore, more elastic tissue and Nakai has proved by investigations in the embryos of different animals that elastic fibres arise first in those parts which require earliest this physical property; e.g. in the large vessels after the heart has begun to beat. The conception of the inter-cellular development of <sup>e</sup>lastic tissue has a definite dependence on this mechanical need of the tissue. In response to the "zugwirkung" there is caused a chemical transformation of the ground substance.

Other writers have seen no reason to ascribe such a significance to the elastic fibres. Goldmann attaches no importance to it and thinks that the new formation is entirely dependent upon the regenerative capacity of the old fibres and to the rapidity of a definite scar tissue.

Dürk<sup>25</sup> (1907) has described a new kind of fibre in the connective tissue and in the vessel wall, which/

which is well brought out by Weigert's stain for medullary nerve fibres. These fibres are all marked by their rectilinear course and rigid condition (telegraph pole-like). Dürk distinguishes in the intima not a membrane but layers of longitudinal fibres with transverse connecting fibres which represent a network with long meshes taking a longitudinal course. This lattice work arrangement of elastic fibres closely surrounds the endothelium and in the capillaries loses itself in the tissue. In the larger vessels radial, rectilinear fibres transverse the media connecting the intima and the externa and Dürk, in consequence of this, describes the coats of a vessel as endothelium and perithelium. The function of these radial fibres is to antagonise the circular muscle fibres. As soon as the contraction of the circular muscle is over, the radial elastic fibres must return to their passive condition and, therefore, automatically dilate the vessel. The presence and function of these elastic fibres lend strong support to the view of the function of elastic fibres expressed in the opening paragraph.

#### (4). MICROSCOPICAL EXAMINATION.

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Sections from each different period both of the incised wounds and abscesses were stained but in this description of the results I confine myself to a series of twelve incisions in the rabbit. These are chosen because they represent wounds in which the reaction has been more or less similar in degree. This description must be taken together with that of the wounds of seven days and five months (Plates X and XI) from human incised tissues kindly given me by Mr George Chiene. In wounds in animals it is very easy to confound the displaced underlying tissue for scar tissue in the search for elastic fibres.

Thirty hours:(slide 91 ). The small intestine had become adherent to the wound and between the two peritoneal surfaces a small quantity of fibrinous exudate had collected. A thin wavy line of delicate elastic tissue can be made out under both the parietal and visceral endothelium. This elastic tissue shows practically no change in staining reaction, and only here and there any fibrillation.

Four/

Four days: (Slide 92 ) A wavy line of elastic tissue with numerous delicate offshoots can be traced along almost the whole length of the peritoneal surface. This line is probably the elastic layer immediately covering the sub-peritoneal fascia and it has been extensively split up and fibrillated. On both sides of this layer a very extensive proliferation of fibroblasts has taken place and amongst these are numerous very fine fibrils. Many of these are so closely applied to the cell as to suggest the appearances of cell border or processes. These delicate fibrils are without doubt the result of the splitting up of the sub-endothelial planes of elastic tissue. At the borders of the wound in the fascial layers numerous clumps and fragments of elastic tissue are found in the condition of homogeneous swelling and in the midst of the granulation tissue delicate elastic fibrils are found. The granulation tissue has probably formed around these broken-off and carried-in fibrils of the original elastic tissue. The staining frequently gives the impression of fibres on the point of dissolution, especially with picric acid differentiation.

Six days: (Slide 93.) Numerous elastic fibres/

fibres are found in the granulation tissue filling the wound cleft. Some are fragments of the old fibres carried into the part, others are delicately stained-many of them on the verge of invisibility. The vessels still have their internal elastic lamina intact but the adventitial sheath is broken up or has disappeared. It is very difficult to say whether these delicate wavy and often stretched fibrils found in the granulation tissue are new fibrils or the dissociated fibrils of old fibres from the adventitia of vessels, hair follicles, and muscle aponeuroses. Their frequent close relation to the elongated fibroblasts suggests more than an accidental laying down of degenerating fibrils alongside.

Six days: (slide 94 ) This preparation shows beautifully the branching off of fibrils from the vessels and the fascial elastic layers into the scar tissue and near the peritoneal surface a distinct layer of elastic tissue with numerous fibrils branching into the tissue on either side. Very numerous straight, wavy fibrils run right up in the scar tissue towards the epidermis. The difficulty arises here again regarding the interpretation of these fibrils. Those branching off from the sub-endothelial/



endothelial elastic layers I look upon as potential new fibres. At present they are only the fibrillated broken up endings of the sub-endothelial elastic layers, but they are still in connection with what seems intact elastic tissue and, however it may be explained, this, according to recent observers, has the power of budding forth new fibres. The fibres - delicately stained and often unstained and refractile - which lie stretched out in the same direction as the fibroblasts in the granulation tissue, must be looked upon as carried-in products which have resisted dissolution. One would like to explain them as new fibrils arisen in connection with these cells but their irregular distribution in the scar tissue seems to negative this supposition.

Nine days: (Slide 195) Only a few very fine fibrils are found in the scar tissue and these are evidently stretching in from the hair-follicles and vessels.

Twelve days: (Slide 75) No fibres are found in the scar tissue. In the deeper parts near the fascial layers are numerous refractile lines and faintly-stained fibrils as in slide 74. They are now on the point of disappearance or again may be potential/



potential new fibres. At the borders of this wound are found some fibres curled up at their ends and a few fibrils from the vessel adventitia stretching into the scar at the sides and deeper parts.

Twenty days: (Slide 96 ) Numerous elastic elements are found especially along the vessels. These are not the delicately stained fine fibrils hitherto described, but possibly fragments of larger fibres which have resisted degeneration. New fibres could not by this time have reached this size and distribution; for these are found scattered more or less through the scar tissue.

Twenty-two days: (Slide 97 ) No elastic fibres can be found anywhere in the scar tissue proper. Scars of eighteen and twenty-five days, where there had been more reaction than in this series, also showed no trace of elastic tissue.

Forty days: (Slide 98 ) The scar tissue forms a small triangular nodule with its apex in the deeper tissues. Only at the margins can any trace of elastic tissue be found. This is in the form of fine granules and delicate fibrils streaming out from the sheaths of the hair-follicles. Numerous giant-cells are seen in this scar tissue but none with any/

any trace of elastic tissue contained in them.

Fifty days: (Slide 98.) Numerous elastic elements are distributed through the tissue in the form of homogeneous clumps or fragments. Near the vessels in the deeper tissues and at the margins of the wound, faintly-stained lines and dots are found. Numerous sections have been stained as control preparations and in all the same appearance was found. These faintly-stained wavy lines thus beginning to penetrate the tissue are probably the first traces of new elastic fibres.

Sixty days: (Slide 99.) A very fine network of elastic fibres, with meshes in longitudinal direction parallel to the surface, lies immediately under the epidermis. Owing to the difficulty in cutting this block, the sections convey a wrong impression of the delicacy of these fibres, for the corium has been compressed by the razor and the fibrils have accumulated in close rows under the epidermis. Yet it can be distinctly seen that numerous long fibrils course parallel to the surface and break up into the minutest branches which encircle cells. From the fineness of this network, its regular arrangement, and delicate staining it must be a new formation. Throughout the whole scar tissue, granules/

granules both coarse and fine are found representing the cross-section of similar elastic fibres and fibrils.

Many scars from eighteen days to three months, when<sup>ne</sup> there had been very severe destructive processes, show no trace of elastic tissue.

The study of these preparations convinces me how very slowly elastic tissue is regenerated and that the main factors in the regeneration are the pre-existing fibres round vessels and hair-follicles. In the scar tissue far removed from the borders or from vessels, at the time when new fibrils are growing in from the borders, new fibrils are being laid down independently. It seems impossible to say whether in relation to the cells or the intercellular substance but it is not inconsistent to assume both forms of development considering how close is the probable dependence of the intercellular substance on the cell. Teuffel<sup>175</sup>\* explains the process in the following way - that the plasma of the cell has the power of forming elastin, and when the intermediate substance becomes secreted from the cell there is communicated to it that same quality.

It is more difficult to understand how the elastic/

elastic fibres arise from old ones. In embryonal development Nakai found that the first fibres arose in the walls of the Pulmonary Artery and Aorta and grew out peripherally. In the development of new blood-vessels a similar process may occur - the cells of the intima to which Jores ascribes a special capacity for forming elastin must transmit this acquired property to their descendants. It is quite possible that elastic fibres, which must share in the life activity of cells in their neighbourhood according to Virchow's view of "cell-territories", have the power of inherent growth in length as they must have of independent growth in thickness.

#### C O N C L U S I O N S.

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- (1) That the elastic fibres found in the granulation tissue in wounds arise from a disassociation into fibrils of the pre-existing fibres. This is brought about by the exudation and the cell-infiltration. The intensity of the inflammatory reaction determines the extent and rapidity of this fibrillation.

(2) /

- (2) That this splitting up is followed by a gradual thinning of the fibrils and a loss of staining reaction. Ultimately the fibrils yield to pressure-atrophy, or solution.
- (3) That homogeneous swollen fragments of single or fused elastic fibres may be found for a long time in the depth of the scar tissue, and at the borders of the wound. It is unlikely that any regeneration can arise from these fragments.
- (4) That the first new fibrils appear in the form of fine threads or spirals with many "cork-screw-windings" as lateral projections from pre-existing fibres at the borders of the wound. That they run in the same direction as the young connective tissue fibres and break up into numerous branches which entwine round cells.
- (5) That it is very probable that in the scar tissue itself a new formation arises in connection with cells or ground substance. The very close/

close relationship frequently seen between the finest fibrils and the cell borders and processes, while the thicker fibrils are farther out from the cell, speaks in favour of the cellular view of origin. The appearance is probably first in connection with the young vessels - analogous to its first formation in the embryo.

- (6) That the amount of regeneration is in inverse ratio to the depth of the wound and therefore of the previous destructive processes; and that the time of appearance of the first elastic fibrils is regulated by the amount of destruction of the pre-existing elastic tissue.
- (7) That in wounds the new formation may be a simple consequence of the organisation of the connective tissue but that the distribution of the elastic tissue in the skin leads one to acknowledge that mechanical forces are factors in the production of elastic tissue.



THE EVOLUTION OF AN ABSCESS.

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I. INTRODUCTORY.

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Many investigators (Councilman, Bardenheuer, Porcile and others) have showed that a suppurative inflammation may under certain conditions be brought about by the action of chemical irritants, such as turpentine, but it is probable that only a few chemical substances have a true chemiotactic power.

BURKHARDT<sup>17</sup>\* by the injection of an extract of bruised muscle substance has also caused an aseptic suppuration. The chemiotactic substances in this case are mostly of an albuminous nature, and act through the setting free of nuclein bodies from the disintegration of the muscle nuclei.

Under ordinary conditions, however, suppuration is caused by the growth of micro-organisms in the tissues and the development of the abscesses in my experiments was produced by the injections of a small quantity of pure broth culture of *Staphylococcus pyogenes aureus* into the subcutaneous tissue of the lateral abdominal wall in rabbits.

I carried out a few experiments by means of/



of the injection of turpentine, but found that the necrosis of the tissues and the spread of the abscess was very great, even with only one or two minims of turpentine. Chemical substances producing abscess-formation have a very marked destructive action and the constructive change is very much delayed.

Suppuration of bacterial origin is, however, admitted to be caused by the production of chemical substances, the toxins of the bacteria. It is the devitalisation of the tissues by these substances that is the most important factor in the extent of the suppuration. Other toxic substances arise, not from the bacteria, but from the decomposition of the tissue elements by the action of the bacterial toxins. Burkhardt has given the name traumatic suppuration to that produced by the formation of chemiotactic-acting substances of albuminous nature, produced, e.g., by the bruising of muscle tissue. These are of comparatively weak power but in their resorption act pyrogenically.

MALLORY<sup>25</sup>\*, in studying the lesions of typhoid fever, came to the conclusion that toxins secreted by bacteria can cause certain cells to proliferate and others to become phagocytic. In a later/

later paper he pointed out that strong toxins cause degeneration of cells and exudation while weak toxins produce proliferation and phagocytosis.

In the introductory chapter I have called attention to the general law that the agent which caused destruction in some way called out the production of those substances which enabled the organism to react and to overcome. The object of my experiments was to produce as small a localised suppuration as possible. In larger abscesses only a partial absorption of the pus takes place and there is no complete substitution. In the smaller the pus cells undergo fatty degeneration and disintegration and are absorbed and the pus is rapidly replaced by a granulation tissue which soon develops into cicatricial tissue.

The injection was very quickly followed by an oedema of the tissues but in course of time this gave place to a small defined swelling and the abscess which ultimately formed was almost equal to the area of the diffusion of the liquid injected. I have considered the histological examination under the following stages:-

i./

- i. From the first grouping of the leucocytes around the cocci to the formation of pus - Plate 45 (fig. a and b.) and 46.
- ii. The Delimitation of the abscess from the surrounding tissue - Plate 47.
- iii. The formation of a distinct membrane of granulation tissue - Plate 48.
- iv. The cicatrisation of this membrane - Plate 49.
- v. The complete absorption and substitution of the pus - Plates 50, 51, and 52.

Plates 53 and 54 represent structural elements of the abscess membrane; Plate 14 (figs. a and b.) - drawings also illustrating these elements; and Plate 13 - a drawing giving a complete view of a section of the wall of an abscess from the normal tissue to the pus area. This latter drawing was from an abscess where there was great delay in the absorption of the pus, and the development of the large mononucleated phagocytic cells was very great.

## 2. HISTORICAL.

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The older works were chiefly concerned with the aetiology of suppuration.

Later investigators have studied the descent of the cell elements which composed the pus and the processes by which the pus area is cut off from the surrounding tissue.

Cohnheim's view was that "suppuration is only an extension of emigration". With few exceptions all authorities have come to the same general conclusion that the pus corpuscles have emigrated from the vessels and that proliferation of fixed tissue cells takes no share in their formation. Deganello has showed that eosinophile blood leucocytes often appear in the suppuration, and frequently also mast-cells and lymphocytes. He holds the origin of the latter two groups to be wholly from the blood. X

It is admitted, however, that it is polymorphonuclear leucocytes with specific granules which emigrate from the blood in large numbers and form the pus cells. Mononucleated cells also emigrate/

emigrate from the vessels but their role and destiny is not so decided as that of the polymorpho-nuclear cells.

BARDENDEUER,\* KIENER\* & DUCLERT<sup>62</sup>\*, and MARCHAND describe large phagocytic cells, which are highly characteristic of the abscess membrane. These by degrees absorb the cell-fragments constituting the pus. All these authors derive these large phagocytic cells from the connective tissue and endothelial cells. They explain the early appearance of such large numbers of these cells on the inflamed area by the anitotic division of connective tissue cells. These cells finally perish and take no part in the formation of the granulation tissue of the abscess-membrane. Later, the regenerative processes depend upon the fixed tissue cells - the connective tissue and endothelial - which divide by mitotic division.

MAXIMOW<sup>82</sup>\* (1905) having studied the cell forms occurring during an aseptic inflammation, has investigated from the same standpoint, the subject of purulent inflammation. He introduced infected celloidin capsules into the intermuscular connective tissue of the rabbit. Sterilised, aseptic, celloidin/

celloidin capsules were used on one side and on the other, the infected capsule.

Maximow found during the early hours the same three cell-forms as in aseptic inflammation: polymorpho-nuclear leucocytes, polyblasts, and the ordinary connective tissue cells. The emigration of leucocytes and polyblasts was much more intense and lasted much longer. The pus was formed in great mass from the former but as the virulence of the process passed off, the small lymphocyte-like polyblasts were found on the borders of the pus mass. These increased in size forming large phagocytic cells and in the tissue around, by the reaction of the connective tissue and endothelial cells, a granulation tissue membrane is formed. The cicatrisation of this membrane arises only from fibroblasts and fitted in between are numerous polyblasts and plasma cells. Maximow derives the plasma cells from emigrating lymphocytes and has showed pictures of lymphocyte emigration in the plasma cell groups. After the absorption of the pus the large phagocytic cells degenerate: a few may become sessile polyblasts.

ROSENBERGER<sup>III</sup>\* (1907) has studied the influence/

influence of passive hyperaemia on the course of a suppurative inflammation. On both sides he introduced celloidin capsules saturated with oil of turpentine and found on the congested side that the early intense hyperaemia, transudation, and migration of leucocytes were followed by an accelerated connective tissue and new-vessel formation.



## (3). STAGES IN THE EVOLUTION OF AN ABSCESS.

## STAGE I: The Formation of Pus.

Six hours: Plate 45 (figs. a. and b.).

Numerous leucocytes infiltrate the tissue and are also collected in small groups around dilated blood-vessels in the subcutaneous tissues.

(Slide 45a). Very many of these leucocytes are so completely filled with cocci that the cell outline is difficult to define. The cocci are chiefly confined to the leucocytes but are also found isolated in the tissue spaces, especially in the fat tissue and between the muscle bundles (Slide 45b). No groups of cocci are seen in either of the sections at this date, and both the isolated cocci and those in the sections stain normally.

There is very marked oedema of the tissues. The collagen bundles are swollen and stain faintly with V.G. (Slide ) many of them are already almost dissolved especially near the collections of leucocytes. Granular fibrin stained grey to black with I.H. is everywhere present in the spaces between the collagen bundles; often the fibrin is in the form of a network of granules. The connective tissue/

tissue cells in the immediate neighbourhood of the leucocyte groups are pale and in every stage of karyolysis: farther away they are less changed but show no evidence of amitosis such as KIENER\* AND DUCLERT\* found at this period. Maximow explains the pictures of amitotic division of cells seen by these observers to the fixation method used.

To a wide extent the vessels are dilated and filled with leucocytes, many of which are fixed in process of emigration.

Numerous small mono-nucleated cells are also distributed in the meshes especially at the borders of the oedematous tissue. They are the same lymphocyte-like cells as in the wound at this period and are nowhere collected in cell-groups.

In the adjoining muscle fibre the capillary supply of the muscle fibre is beautifully brought out (Plate 6.fig.a.) and illustrates how easily and rapidly the muscle fibre may be penetrated by leucocytes and other cells. CARTER\* and MEIGS\* have described capillaries in the interior of cross-striped muscle fibres. This may also account for the rapid penetration and dissolution of the muscle substance/

substance. THOMA<sup>127 (1907)</sup>\* however, has pointed out that this appearance of vessels within the muscle fibres may be due to the capillary lying in the thickness of a membrane formed by the fusion of the Sarcolemma of two muscle fibres. He has found apparently-ramified fibres with elongated meshes in the cross-striped muscles of many vertebrates. In this way would be explained the transmission of energy even if the individual muscle fibres are much shorter than the whole muscle. It is quite possible that the leucocytes may penetrate the unbroken sarcolemma sheath as parasites penetrate the envelope of a red blood cell.

The muscle fibres already show in many parts a dissolution by the action of the leucocytes or the toxins of the bacteria. Between the muscle fibres both isolated cocci, and isolated leucocytes containing cocci are found. In this section one or two endothelial cells in the intermuscular capillaries were found containing cocci but the pictures were not absolutely convincing.

In the fat lobules there is a very marked heaping up of leucocytes and these are nearly all densely/

densely packed with cocci. Cocci are also found isolated between the fat cells, which, even distant from the leucocyte groups, are outlined by rings of leucocytes containing cocci. This shows how rapid is the infection of the fat tissue in a wound.

The peritoneal endothelium when retained stains very faintly and is swollen, presenting often a beaded border to the subendothelial oedematous tissue (Slide 313. ).

Twelve to Eighteen hours. (Plate 46). The intensity of the reaction depends naturally on the virulence and number of the organisms injected, and the resistance of the tissues. There is, therefore, great individual variety in the intensity of the process. In one of my animals killed after twelve hours there is greater reaction than in one of those killed at eighteen hours, but in the second animal killed at eighteen hours there was found a progressive intensity in the changes.

Definite pus had not yet formed but the tissues are infiltrated with leucocytes. The densely infiltrated area extends from the corium to the deeper muscles. These had undergone coagulation necrosis/

necrosis & more in parts surrounded & eaten away by leucocytes. In other parts the reaction had not extended beyond the deep fascia covering the muscles.

The fibrin network, containing remains of the dissolved collagen fibres, is very visible at the borders and remains of collagen bundles can be recognised amongst the dense masses of cells. The vessels in these areas are so blocked with leucocytes that under low power they cannot be distinguished from the surrounding infiltrated tissue. The leucocytes in the centre of the denser areas are mostly degenerated; the nucleus staining diffusely and the protoplasm dissolved. Pyknotic cells are not marked. Numerous cocci are found in the leucocytes in the infiltrated area but few in the peripheral parts. The cocci in the cells stain a little more diffusely than normally and appear swollen. Only a few isolated cocci can be found.

At the periphery the vessels are also dilated, surrounded by leucocytes in active emigration and here also are found numerous mononucleated cells. Some of these are fixed in the tissue spaces in amoeboid movement (Slide 478). The connective tissue cells are swollen but show no evidence of amitosis nor mitosis.

Twenty/

(218. 279-280.)

Twenty-four hours: The centre of the dense area has now become a vitreous mass in which we find necrosed tissue cells (faintly recognisable), red blood cells, and leucocytes. The cocci in the centre of this mass are nearly all degenerated elements; on the borders leucocytes with contained cocci can be made out. Around this degenerated central mass the oncoming leucocytes collect endeavouring, as it were, not only to engulf the cocci but to shut them off from the surrounding tissue. Between the central degenerated cells and these well-stained cells are all transitions, and every form of degeneration can be made out. Pyknotic cells are now abundant but the pyknotic nucleus stains diffusely. On the borders Karyorrhexis is more evident and as the fresh leucocytes reach this area, many seem to undergo Karyolysis. Chromatin particles, isolated and in masses, are found everywhere.

The first leucocytes in their defence of the organism have been killed by the toxins of the bacteria (leucolysis) and with their disintegration a proteolytic enzyme is set free, to which is now supposed to be due the liquefaction of the tissues.

The softened and liquefied tissues with these/

these dead leucocytes, dead red blood cells, and dead tissue cells constitute pus. X

As new leucocytes are continually emigrating from the periphery to the borders of the pus, we still find cells which stain normally and which contain a few cocci. I have never found cocci definitely in mononucleated cells in the pus borders, except those which might be explained by phagocytosis of leucocytes containing cocci and the subsequent digestion of these within the mononucleated cells with the setting-free of the cocci.

Maximow states that their presence in mononucleated cells indicates a non-virulent organism.

In the peripheral area the tissues are reacting to the irritant. The vessels are dilated and surrounded by numerous leucocytes and lymphocyte-like cells: the connective tissue cells show signs of awakening: the endothelial cells of the vessels are swollen, and granular, and show a darkening of their nucleus. In the cells of the stratum germinativum, very numerous mitoses are found. ✓

SUMMARY/



SUMMARY OF FIRST STAGE:

Emigration of leucocytes in great numbers around the cocci with marked phagocytosis by the leucocytes. Degeneration of these in great numbers. Necrosis and liquefying of the tissues. Most of the cocci already taken up by the leucocytes or free in the pus.

Commencing reaction in the peripheral zone.

## STAGE II. Delimitation of the Abscess.

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### Thirty Hours to Two Days : (Plate 47.)

Under the low power the pus mass is clearly defined from the surrounding tissue (Slide 28/ 30 hours). Degenerating leucocytes, with numerous chromatin particles irregularly distributed among them, border a central, closely-compressed, structureless mass which stains diffusely and reveals only that cell and tissue elements originally composed it. Around the border of degenerating leucocytes is a zone consisting of fibrin, normally-stained leucocytes, a few mononucleated cells and pale swollen fibroblasts. In this zone may lie structureless clods of muscle substance, surrounded and penetrated by leucocytes and fibrin (Slide 28/ ).

This zone of demarcation, where the tissue elements are still recognisable, intervenes between the central, structureless area - the pus and an inter-zone of reaction, which again passes peripherally into normal tissue. This zone of reaction is the first stage in the formation of a granulation-tissue membrane. In it numerous dilated capillaries pass from the normal tissue towards the central mass. These are filled with/

with red blood cells and leucocytes and are surrounded by similar cells, fixed in amoeboid movement. Those migrating leucocytes, as so often before, give the impression of having a definite purpose in their activity - to reach the area where they are needed or, seem as if drawn there by some irresistible chemio-tactic force.

Between these dilated vessels are numerous fibroblasts arranged parallel to the surface of the pus mass. Many of these cell-forms on cross-section are very difficult to distinguish from cells which might be "mononucleated cells" (slides 47-314). The protoplasm is reticular and swollen from the absorption of the exudation. The nucleus of the mononucleated cell has usually the indentation of the nucleus and the folding of the membrane as distinguishing marks. Numerous mitosis are present amongst these connective tissue cells (Slides 283-285). The capillaries also show very numerous mitoses (Slide 47<sup>314</sup>). On cross-section of the vessels this zone resembles a meshwork of dilated capillaries with swollen endothelial cells, many of which are dividing. When this zone lies in fat tissue (Slides 47 and 284<sup>315</sup>: 2 days) this appearance is much more evident. Dilated capillaries amongst branching fibroblasts, numerous leucocytes, and/

and a few eosinophile cells.

A few mononucleated cells surround the vessels bordering the fibrin zone - some already showing phagocytosis to red cells (slide 281) and leucocytes (slide 282). This is the first evidence of those phagocytic processes which will take so great a share in the absorption of the pus. (36 hours). It becomes very evident in two days ( Slides 283-285). Maximow states that the appearance of mononucleated cells (polyblasts) in considerable numbers indicates a beginning retrogressive metamorphosis of the inflammation. When the cocci are actively on the increase we get only leucocytes emigrating to the pus mass. They are the first line of defence of the organism against the cocci and as long as the cocci increase the number of leucocytes killed in the battle increases and add to the amount of pus. In the battle, however, they have destroyed many of the cocci: partly directly through phagocytosis and partly through the elimination of certain substances which act injuriously on the cocci. The cocci in these sections are found chiefly within the leucocytes, and very many show signs of degeneration - often staining diffusely with safranin. A few isolated cocci are still found ( Slides/

(Slides III: 30 hours: and 36 hours).

As the cocci disappear or are weakened we get the lymphocytes appearing. These soon enlarge into phagocytic mononucleated cells, and together with mononucleated cells derived from the proliferation of endothelial cells, form the second line of defence. The first-appearing mononucleated cells on the pus border are probably also killed and can not be distinguished from pyknotic leucocytes but others soon come up to take their place.

STAGE III. Formation of a Granulation-  
tissue membrane.

Three to Six days: (Plate 48)

As a result of the reactive phenomena commenced in the last stage we have a granulation-tissue membrane formed around the pus mass. This is early differentiated into two layers - an outer which passes into the normal tissue, and an inner surrounding the pus. Owing to the great importance of this stage very numerous experiments were carried out for the period between three and six days. In several of these the pus came almost to the surface and in two cases opened into it.

Cocci are numerous beneath the surface epithelium and stain well.<sup>1/3</sup> In the deeper tissues they have lost affinity for the Gram's stain and on the borders of the pus degenerating leucocytes with safranin-stained cocci are seen in large numbers. No cocci could be found in endothelial cells.

The central pus mass in many cases is still surrounded by a fibrin layer (Slide 290.) in which lie numerous leucocytes and the remains of tissue elements which have escaped necrosis.

Outside/

Outside this is a very cellular and vascular layer in which end numerous vascular loops and buds surrounded by large mononucleated cells and leucocytes. This layer is becoming differentiated as the inner zone of the abscess membrane. The young vessel shoots reach to the periphery of the pus and form a beautiful branching network. Very numerous mitoses are found in the endothelial lining of these young vessels (Slides 316-320, <sup>287</sup>/<sub>292</sub>). On cross-section of the vessels at this period we have extremely frequently signet-ring appearances and nucleated buds as in Plate 4 (figs. b. and c. )

Around these budding vessels are closely compressed cells nearly all of which are mononucleated and polymorphonuclear (Slides <sup>298</sup>/<sub>290-293</sub> ). A few fibroblasts may be seen but many are probably hidden by the numbers of other cells. As we near the pus border the mononucleated cells enlarge and become vacuolated and on the borders of the pus contain numerous cell-inclusions. The leucocytes are still on their way to the pus area and are fixed in all phases of movement. The larger vessels contain numerous mononucleated cells, some flattened against the wall. I have not found undoubted pictures of emigration/



emigration but am convinced that emigration of lymphocytes is adding its share to the numbers of mononucleated cells found in this inner zone.

The outer zone which by six days has become clearly defined contains widely dilated vessels, filled with leucocytes and mononucleated cells. The vascular network is, however, not so abundant and gives the appearance of being the source from which streams into the inner zone the extraordinarily rich network of vessels which characterises it. The vessels too are arranged more regularly, all radiating at right angles to the circumference of the pus mass, and at the border of the inner zone break up into branches. Between them are found very numerous fibroblasts arranged irregularly. In some parts they are parallel to the pus mass and in others are slanting or parallel to the vessel walls, to which they seem to have applied themselves as an outer layer.

On cross-section of vessels in this outer zone we find numerous vessels with two or even three layers of cells lying superimposed, or almost so, on one another. These all stain similarly (Slide 37. M.G.P.). The close apposition of these cells seemed to indicate that they have arisen by mitotic division of/

of the endothelium. Similar cells lie immediately surrounding the vessel and the apparent transition from these to the surrounding fibroblasts is very evident. It is very difficult, however, to be convinced that these superimposed cells are endothelial cells passing out into the tissue and becoming fibroblasts. (On the relation of endothelium to fibroblast formation see page 91 ).

By the gradual re-arrangement of the fibroblasts at right angles to the vessels and by their further differentiation this outer zone will form a capsule enclosing the pus mass and phagocytic cells.

This outer zone merges gradually into more normal tissue in which are seen both in longitudinal and cross-section, the larger vessels which gave origin to those perpendicular vessels in the outer zone. Around these vessels are beginning to be differentiated plasma cells, which already ( 4 days, Slide 316. ) show specific characters. They are few in number as yet and not quite typical.

In certain parts the vessels both here and in the outer zone are surrounded by numerous cells with large granules. Muir's granule stain (E.M.B.) and I.H. show them to be true eosinophile cells.  
The/

The vessels show very few within their lumen and an explanation of such large numbers as in some of my preparations is very difficult to find. A possible explanation is that the ordinary polymorpho-nuclear leucocytes have taken up the red blood cells and from their disintegration result these dense masses of granules. In favour of this theory is the fact that both granules and red cells stain greenish with M.G.P.<sup>3/6</sup> and black with I.H.<sup>2/9</sup> but against it is the fact that no transitions are seen.

#### SUMMARY.

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The essential characteristics of this stage are in relation to the two zones into which the tissue surrounding the pus is now divided. In the inner zone we find intense phagocytic activity and numerous young wide<sup>ves-</sup> cells. The latter will aid the absorptive processes by carrying to the part substances which will help the cells to carry out their functions, and also by carrying from the part dissolved substances. In the outer zone we have the proliferative activity of the connective tissue cells which is to result in encapsulating the pus, and thus form the third line of defence of the organism against infection.

STAGE/

STAGE IV. The Cicatrisation of the Mem-  
brane and Absorption of pus.

Eight to Twelve Days.

Plate 49 shows very clearly the two zones at this period for the same two processes are at work. There are great variations in the time of the encapsulation. If, for example the abscess at its height had formed a swelling about the size of a nut the definite encapsulation is distinct in ten days. (Slide <sup>296</sup> 297<sup>322</sup>). I take as examples two abscesses of eight days and ten days. In the former the swelling was very defined but very limited. Sections through the centre of the little nodule showed the pus area bounded on one side by dense fascia, where there had been little reaction, and on the other numerous vessels radiated towards the pus mass, accompanied by large phagocytes which had already almost removed the pus. In the outer layer of this border the cicatrisation was going on (Slide <sup>298</sup> 299<sup>321</sup>). In the abscess of the ten days there was a definite fibrous capsule around the pus mass.

The cicatrisation of the outer zone is gradually extending in breadth as it encroaches on the/

the outer part of the inner zone. The fibroblasts have their long axes arranged circumferentially to the pus mass, and are laying down numerous concentric fibrils. Mononucleated cells, many of which are plasma cells, are found alongside the vessels. The inner zone is now less definitely marked off owing to the extension inwards of the fibrillated laminae and fibroblasts.

As we pass inwards numerous fibroblasts are now found between the vessels. The mononucleated cells may still be so numerous as to hide from view all the other elements. They increase in size as the pus mass is reached, and form there layers of large endothelial cells with abundant cell-inclusions and many vacuoles. The young vessels penetrate amongst these cells right up to and even into the remaining pus. Numerous mitoses are found in the endothelial cells of these vessels. The pus area is gradually lessening in size by a double process of absorption of its contents and of shrinking of the capsule which surrounds it. No Gram-positive cocci can be found: but the large phagocytic cells at the borders of the pus contain very numerous swollen cocci staining with Safranin. These are found along with the/

the cell-debris of leucocytes.

In the perivascular spaces of the normal tissue bordering the capsule are beginning to appear numerous cells, which show all transitions from lymphocytes to plasma cells. In the tissue spaces surrounding these vessels and reaching into the outer and even into the inner zone, plasma cells are also found in small numbers, frequently in rows of three or four in a tissue space or alongside a vessel in longitudinal section.

These are the first signs of the so-called "small-celled infiltrations" which in more chronic abscesses and in numerous pathological exudations are so frequent. Numerous eosinophile cells are also present both around vessels and distributed irregularly in the tissue. Dilated lymphatics are found in the outer border of the capsule. These contain numerous smaller and larger forms of mononucleated cells, many of them enclosing cell-debris. (Plate 54).

SUMMARY./

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SUMMARY.

Under the influence of the vascularization of the inner zone the large phagocytic cells are actively causing the absorption of the pus, and owing to the cicatrization of the outer zone fibrous laminae are being laid down closer and closer to the pus mass. In the outer layers are found numerous plasma cells, and these here and there are grouped with numerous lymphocytes in perivascular infiltrations.



STAGE. V. Complete Absorption and Sub-  
stitution of Pus.

Twelve to Twenty Days.

Small abscesses which have not opened externally will during this period be completely absorbed. (Slide 179: 223: 15 Days). The phenomena of absorption have begun gradually as soon as the first mononucleated cells have reached the pus border. The gradual ingrowth of the vessel shoots, accompanied by increasingly numerous & active pus phagocytes, continues till the whole of the pus mass is removed, and there remains only a mass of large vacuolated cells with delicate vessels ramifying among them. (Slides 222-223) Gradually fibroblasts penetrate still further in from the former outer zone and fibrous laminae are laid down till in process of time a scar tissue takes the place of the pus.

Among the vacuolated cells are found numerous plasma cells which have reached this central area from the perivascular infiltrations. The numbers of Plasma cells are not nearly so numerous either in the wall or in this group of vacuolated cells as in more chronic abscesses where the substitution has been/

been slow. (Compare slides 323 and 325).

The slides illustrating this stage of complete absorption are fifteen days and seventeen days. In both, small areas of completely absorbed pus with substitution by vacuolated cells are found. In the tissue spaces between the concentrically arranged fibrous laminae which surround these areas are numerous plasma cells. Other mononucleated cells are becoming sessile especially around the vessels. Some of these are elongated and even spindle-shaped but can be distinguished from the fibroblasts by their darker nucleus and more defined, smaller cell-body. (Plate 14 ).

I have no specimens illustrating the complete replacement of an original, single small pus area by scar tissue. This would have completed the series I have just described of small abscesses from their formation to the substitution of the pus by scar tissue. My preparations stop short at the complete substitution by vacuolated phagocytic cells, amongst which there are now no pus cells. During the final phagocytic activity of these cells all the component parts of the pus are absorbed. The chromatin particles arising from the disintegration of the cells/

cells, the last traces of the cocci in the cells are seen lying in vacuoles. Then they too finally disappear and as the cell-inclusions disappear, the protoplasm of the pus-phagocytes obtains a fine honey-comb appearance. The final stage of the formation of scar tissue I have traced in the small foci around larger abscesses e.g. (Slides 305-307. 51:50 days). Delicate vessels pass in amongst the vacuolated cells. These are accompanied by fibroblasts and small mononucleated cells like those which first emigrated. Plasma cells are also found amongst these. Soon a vaso-formative network bridges the small cavity. The great mass of the vacuolated pus phagocytes disappear, I think, by Karyolysis (a few become pyknotic). Others are removed in the lymph stream. I have seen no evidence of transition forms between these large vacuolated cells and sessile polyblasts and think that, their function fulfilled, these cells so characteristic of the inner zone of an abscess-membrane are removed altogether. The fibroblasts proliferate, fibril formation occurs, the vessels dwindle, and a cellular scar tissue takes the place of the pus.

## ABSORPTION OF PUS IN LARGER ABSCESSSES.

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The pus in larger abscesses is not completely absorbed but remains encapsuled by fibrous tissue. From the inner surface of the abscess membrane vascular buds project into the pus mass. As these contain all the elements of granulation tissue they increase in length and thickness until the abscess cavity, if not too large, is divided up into compartments. Each vascular partition is bordered by large phagocytic cells as in the smaller abscesses, and the two-fold processes of absorption in an inner zone and cicatrisation in the outer, go on till these small foci of pus are substituted by vacuolated phagocytic cells and finally scar tissue.

In an abscess such as that in Plate 52, one could trace all transitions between abscesses with vascular buds just beginning to penetrate into them (Plate 51 and Plate 52 fig. 1 c ) and those where the partition had completely crossed the cavity. Many of these small foci of suppuration have probably arisen around original small collections of cocci: but many must undoubtedly be due to the dividing-up of/

of larger foci. Plate 52 fig. 1. c. shows a small focus with vascular buds on opposite sides which will soon meet. Plate 52 fig. 2 (a higher power view of fig. 1.) shows the network of vessels forming these buds, and the numerous phagocytic cells bordering the pus. It is on the borders of vascular buds that the pus phagocytes reach their highest differentiation. Plate XIV. fig. 1. shows a few of these cells stained with I.H. They are very large and globular and contain in their cell-body: (1) degenerated or intact leucocytes or the products of their disintegration. (2) numerous granules which stain with Saffranin and are colored red with M.G.P. and (3) numerous vacuoles.

Maximow regards these granules as products of the assimilation by the cell of dissolved albuminous substances which are then precipitated in the protoplasm. The vacuoles also, Maximow looks upon as fat particles - not a direct absorption of fat particles but the expression of the digestion of the products of degeneration and to be considered in the sense of a granular fat synthesis (Arnold). Plate XIV shows very beautifully these vacuolated phagocytic cells. One is seen in Mitosis.

In/

In the larger partitions between these foci are found very numerous groups of beautifully developed Plasma-cells (Plate XIV. 6). In small foci replaced by vacuolated cells, there is always in chronic abscesses a very marked penetration of Plasma cells (Slide 326 ) and in these partitions also is brought out more typically than elsewhere the possible transition between sessile mononucleated cells and fibroblasts. In one field (Plate 14 fig. a. ) are seen several cells which represent all transitions between the round lymphocyte-like cell with narrow edge of protoplasm, the larger mononucleated cell with a clear area developed in the protoplasm, and lengthening out of these to form cell-forms of spindle-shape and even with processes. All transitions between the clearer nuclear structure of the fibroblast and the denser structure of these elongated mononucleated cells can also be seen. Around blood-vessels in longitudinal section the similarity and at the same time the differences are brought out even more clearly. I have seen nothing in my study of these processes to indicate that these elongated mononucleated cells develop into fibroblasts.



THE STRUCTURE OF GRANULATION TISSUE AND THE  
NEW FORMATION OF BLOOD VESSELS.

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The term "granulation tissue" is now applied to any cellular proliferation but it was originally given to the structure known as a "granulation" which appears on the surface of an open wound.

The structure of wound-granulations is decided by the arrangement of the newly-formed vessels. To every raised point there corresponds a vessel stem which arises out of the depth and falls into numerous branches dividing dichotomously and at acute angles. These branches run upwards in a fairly straight course giving off numerous lateral branches which bend as loops near the surface. The parts bent like a loop appear wider than those which take a straight course. The latter have adventitial spindle-shaped cells closely applied to their outer wall.

It is to the arrangement of the tissue elements in one of these "granulations" that the following paragraphs will be devoted. A series of plates illustrate the structure of the different layers - those/



those have been drawn or photographed from slides -. Plates 15 and 16 show the surface, intermediate, and deep layers of granulation tissue. Plate 17 one of the capillary loops formed by the union of two lateral branches and giving off other branches towards the fibrin surface. Plate 1. reveals in detail the structural elements from preparations stained with M.G.P.

For purposes of illustration and description I have chosen granulation tissue of six days. <sup>Slides</sup> 1. a. b. c. d. e. 2. At this time the cellular proliferation and new-vessel formation are in full sway and the different layers are more typically developed than at a later date.

If we follow the tissue from the wound surface to the deeper tissue we can distinguish the following layers which I name the Surface, Intermediate, and Deep layers. This last borders the normal tissue out of which arise the vessels, whose branching anastomosis forms the framework of the granulation.

#### I. Surface of Fibrin Layer Plate 1. (fig.1.)

This is composed of beautifully reticulated fibrin filaments/

filaments in the meshes of which lie numerous cells (Plate 1 ). The amount of the fibrin poured out and the proportion of the cells vary very much. Leucocytes are in large proportion in the meshes but mononucleated cells of various sizes and fibroblasts are also present. The fibroblasts have beautiful star-shaped or ramified forms or may appear like large antler cells (Slide 1(d) ). Some have a rounder appearance but can always be easily distinguished from the mononucleated cells by their clearer, oval nucleus and the presence of several nucleoli. The more reticular protoplasm stains a deeper red with the pyronin. The mononucleated cells have usually an indented nucleus, seldom more than one nucleolus, and this only in the larger forms and a vacuolated protoplasm with frequent cell-inclusions. (Plate 1).

Two questions arise in relation to these fibroblasts. How have they reached this layer and what is their function there so early? They must have migrated there from the deeper tissues where actual proliferation is going on or have been carried thither by the upward flow of lymph. In their migration they may use the fibrin filaments as guiding lines. But what is their significance there? Their/

Their long interlacing processes form a very intricate network amongst the fibrin filaments. These long branching processes seem now to enter into especially intricate relations with the young vascular buds which are found so abundantly bordering this fibrin layer. The end of their long processes spreads out and attaches itself to the endothelium forming the young vessel wall. Processes from several fibroblasts may attach themselves to the same bud. Their direction being, as it were, a direct continuation into the fibrin layer of the young vascular buds. It is difficult to resist the conclusion that these cells are later incorporated into the vessel wall as endothelial cells. They certainly serve the young vessel as a guide and possibly as a motive power.

II. The Intermediate Layer may be divided into two portions - an upper, more superficial part and a lower, deeper part. These two cannot very easily be differentiated. The fibrin layer merges into the next by a grouping of the elongated fibroblasts (Plate 1 ). The upper portion is characterised by the presence of numerous capillary loops (Plate 17) and/

and buds (Plate 15 fig.2) between which lie numerous fibroblasts irregularly arranged. The fibroblast processes frequently anastomose. Cells, similar to those which deeper in this layer form the capillaries, are being apparently arranged to form a capillary endothelial wall. It is impossible to distinguish between these cells and many of the fibroblasts by which they are surrounded: both have an oval clear nucleus and true nucleoli. In both the reticulated protoplasm stains deeply with pyronin. Where the endothelial cells have definitely formed a capillary wall the protoplasm seems to stain more deeply but the difference is only relative. Frequently the endothelial cells show distinct granules with Muir's stain.

Numerous mononucleated cells, red blood cells, and leucocytes are found between the vessels. If the surface is under special irritation, the number of the leucocytes is very marked. They may be collected into circumscribed groups around which are gathered very large mononucleated phagocytic cells with very varied cell-inclusions. Numerous giant-cells are found in this layer gathered around hairs and dust particles &c. There may be seen frequently the stages of formation of these giant-cells by a fusion/

fusion of the smaller mononucleated cells. As these are incorporated into the body of the giant cell true nucleoli are developed. If the cell-content of this layer is not too great the vessel loops and buds stand out very distinctly under low power.

Numerous fibroblasts are irregularly grouped around them and the elongated stretched out processes of cells in the fibrin layer are attached to them.

This portion of the intermediate layer merges into the next by a very gradual transition. The endothelial cells are now definitely grouped to form capillaries, which all take a radiating direction (Plate 16 fig.3). Between these the fibroblasts are becoming definitely arranged with their long axes parallel to the capillary wall. Many have to a great extent taken in their side processes and still others lie close to the outer surface of the endothelial wall. The latter are united to one another by their long processes and give the appearance of forming a second wall to the capillary exactly similar to the endothelial wall. The cells immediately outside the vessels are beginning to show fibril formation in the layer of protoplasm not in relation to the vessel wall. As this increases the cells seem/

seem to separate a little from it - leaving a perivascular space in which are sometimes found smaller elongated cells with darker nuclei.

The intermediate layer still contains fibrin even in the deeper portion, also numerous leucocytes and mononucleated cells. The leucocytes diminish in number as we reach the deeper tissues.

III. Deep Layer. This borders the normal tissue and the spindle-shaped fibroblasts are arranged in its deeper part at right angles to the vessels (Plate 1. and Plate 16 fig.4). The transition between this zone and the previous one is marked by cells running diagonally. Between the fibroblasts lie numerous mononucleated cells, some of which are becoming elongated especially those in relation to vessels. This zone contains few leucocytes and seldom phagocytes or giant-cells.

The young vessels passing up from the foundation layer give off anastomosing branches in the intermediate layer and in the upper part of the latter give rise to vascular buds. This intermediate zone is a perfect network of capillaries. As we near the deeper tissues the anastomosing branches can/

can be recognised to have undergone involution and have become endothelial cords. Greenfield has pointed out that the channels of vessels in organising tissue, which fall into disuse, are closed by a natural endarteritis.

The cicatrisation of this tissue is carried out as in granulation tissue in the healing of wounds. In all the three zones numerous mononucleated cells are found, a few of which become in the scar tissue sessile adventitial cells. Plasma cells are beginning to be found around the vessels in the deep layer and in the normal tissue. In all the layers numerous mitoses are found both in endothelial and connective tissue cells and a few in mononucleated cells. I have been unable to attribute any distinctive characters to the mitoses of the various cells.

The New Formation of Blood-Vessels is one of the earliest and most essential steps in all new tissue formation.

Cornil and Ranvier hold that spindle-shaped cells may arrange themselves in rows to form an inter-cellular canal - the lumen of which is later connected/  
ed/



connected with the lumen of pre-existing capillaries.

The so-called intercellular formation of post-embryonic life is a process of "Budding". The first appearance is a solid more or less conical bud, with a long tapering process, on the outer surface of an endothelial cell. This solid "bud" may be hollowed out from the lumen or the solid "bud" may become a nucleated mass of protoplasm which differentiates itself into separate cells. The lumen of the capillary is pushed out into this bud, the cells of which re-arrange themselves to form the wall. The bud, either hollowed out or canalised, joins with another bud by means of their processes and the protoplasmic bridge thus formed becomes a capillary loop.

The new capillary wall soon becomes thickened by proliferation of the endothelial cells or by apposition of new cells. The method of the production of the elastic and muscular coats is little understood.

In no part of this subject have I found it more necessary to recognise the limits of the usefulness of my material than in regard to blood-vessel development. Again, on no subject have my preconceived ideas been so hazy and therefore it has been easy to interpret certain appearances as the recognised/

recognised methods of development. Blood-vessel loops formed by the union of two lateral branches have been easily recognised in many of the preparations from which I have described the structure of granulation tissue. No more perfect picture could be found than that illustrated by Plate 2 and Plate 17. Again the collection of the protoplasm of the endothelial cell at the point of growth and the sending out of thread-like processes in different directions - these have been the most frequent appearances I have seen in these preparations. I have often found the nucleus in this protoplasm mass, and mitosis of the nucleus. A collection of endothelial cells has often been found at the ends of these vessels with fine threads proceeding from them, or sometimes this group of cells is continued by a single or double nucleated row some of which may show mitotic figures. In the granulation tissue on an open surface I have thought I have found a protrusion of the membrane of flattened endothelial cells - either a simple thin membrane with only protoplasm, and nucleus only at base on one or either side. (*Slides. 115-135 : 257-287. : 318-321*)

In wounds the appearances which I have interpreted as the first signs of a new formation of vessels/

vessels are those represented in Plate 4. Fig.1 shows an endothelial cell in mitosis protruding out from the vessel wall. The earliest stage of this would be a thickening and protrusion of the wall at that point and then the division of the nucleus. Later division of the nucleus would result in conical nucleated masses such as are seen in figs.2 and 3 and which could scarcely be interpreted as surface view of the endothelium of a vessel. ~

## CONCLUSIONS.

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The object of my study has been to give a detailed description of the histological picture of wound-healing. I can, therefore, best further this object by endeavouring briefly to summarise the stages in the healing of an ordinary incised wound. It will be evident that in doing so I cannot qualify my statements by referring to the many incidental factors which varied the sequence of the phenomena.

I (a). At six hours the area of solution of continuity resulting from the incision is occupied by granular fibrin. This is bordered by a zone made up of degenerating tissue remnants, connective tissue cells, and endothelial cells which show Karyolysis. In this area the polymorphonuclear leucocyte is abundant. On the second day lymphocytes and longer forms of mononucleated cells are found in this area bordering the fibrin—the zone of repair. In four days young blood-vessels, fibroblasts, and mononucleated cells are seen penetrating into the fibrin and breaking it up, and the fibrin strip has been replaced by those/

those elements on the sixth day.

(b). The cells of the inflammatory exudate. At six hours in the zone of repair the polymorpho-leucocyte alone is apparent. In the zone of reaction the preponderating cell is the polymorpho-leucocyte. In addition there are found a few lymphocytes and larger lymphocyte forms. The lymphocytes found here have practically all come from the blood stream and evidences of their emigration are apparent. At twelve hours the numbers of the mononucleated cells in the zone of reaction is much increased, and at eighteen hours they appear to be as abundant as the polymorpho-leucocytes. These latter are still the preponderating cells in the zone of repair. Here the mononucleated forms are seen occasionally to be vacuolated - this in my opinion is a preparation for functional activity.

(c). Up to eighteen hours none of the cells in the inflamed area show evidence of proliferative activity. The first evidence of this I observed at twenty-one hours. Mitosis was found in both tissue and endothelial cells at this period. The earliest evidence of reaction on the part of the fixed/

fixed tissue cells is visible, however, at six hours. The endothelial cells of the capillaries are swollen and granular. The connective tissue cells show no distinct reaction. At twelve hours the endothelial cells show the same change more intense in degree; this is shared in by the connective tissue cells. These cells are characterised by the granularity of their protoplasm, the cell body is more defined and separated from the collagen bundles. At eighteen hours no mitosis is visible in endothelial or connective tissue cells. In both, however, a re-arrangement of the chromatin network of the nucleus is visible. This network is denser, coarser, and more evident, such as is seen prior to cell-division.

II. The second stage: (a). From twenty-one to thirty hours there is seen the active formation of mononucleated phagocytes in the zone of repair. These are derived mainly from the endothelial lining of the small blood-vessels. These cells swell up so as almost to occlude the lumen of the capillary, and appear to become loose in it. Around the vessels cells similar in appearance are found/

found and differing in size from the emigrated lymphocyte. Very numerous mitoses are found in the endothelial cells of these vessels. No evidence is yet present that this endothelial reaction is concerned with new formation of blood-vessels.

The first evidence of blood-vessel formation is seen at thirth-six hours. This appears as the projection from one side of the vessel of a nucleated mass of protoplasm, which in later stages forms a multinucleated mass. At sixty hours to seventy-two hours this becomes lengthened out, cells become differentiated, and the canalised bud forms a young capillary shoot.

During this second stage the phagocytic mononucleated cells, derived by emigration from blood by proliferation of capillary endothelium are in great numbers in the zone of repair; the connective tissue cells have entirely disappeared. In the zone of reaction the mononucleated cell is the preponderating cell. The connective tissue cells are also increased in number and show mitosis.

III. The Third Stage. From thirty-six hours - four days - this stage of fixed tissue cell reaction preparatory/



preparatory to restitution shows connective tissue cell proliferation at its height. Young capillaries are seen forming loops by the anastomosing of lateral branches. This stage is also characterised by the presence of fibroblasts in the zone of repair and in the fibrin to which areas they have migrated from the zone of reaction. In this latter area the fibroblasts show very numerous mitoses, but no evidence of fibril formation.

#### IV. The Fourth Stage. From four to seven days.

This stage of fibril development is characterised by the disappearance of fibrin from the zone of repair. The blood-vessels are seen forming rows of endothelial cells stretching across from side to side of the wound. These are accompanied by fibroblasts which are arranged parallel to their outer surface.

The earliest evidence of fibril formation is seen at four days. This appears as a fibril inside the cell-protoplasm. At four days few evidences of ramifying processes from the cell are found. These are, however, apparent at five days and give the impression of unravelling into  
a/

a sheaf of radiating fibrils. These appear to become isolated from the cell and form the intercellular fibrillary ground substance. No evidence of the definite formation of fibrils in the extracellular tissue has ever been noticed by me.

The mononucleated cells are much fewer in number. More that are present are vacuolated. No evidence of the settling down of these to form sessile adventitial cells is apparent.

V. Fifth Stage: seven to ten days. The stage of condensation of the fibrous laminae is characterised by an alteration in the fibroblasts in the zone of repair. They are now arranged regularly with their long axes at right angles to the long axes of the young blood-vessels and extend across the wound. They also show active formation of fibrils and have abundant intercellular fibrillary ground substance between them.

The young blood-vessels, especially in the deep part of the scar tissue, have also undergone an alteration. They are now seen to be frequently occluded and converted into cords of endothelial cells. This change, in my opinion, is caused by an endothelial proliferation and the contraction/

contraction of the scar tissue - resulting from the extravascular formation of young fibrous tissue in increasing amount.

A gradual increasing condensation of the scar tissue is apparent. Such open spaces as exist are seen around the larger, pervious vessels, and in these areas a few mononucleated cells and an occasional polymorpho-nuclear leucocyte remain.

In the evolution of an abscess the changes are identical in their fundamental characteristics to those in repair of wounds but are modified by the intensity of the irritant. The differences are a liquefaction of the tissues and the accumulation in great numbers of the cells of the inflammatory exudate which go to form the mass of the purulent fluid.

The polymorphonuclear leucocytes are called out in great numbers and already in six hours show very intense phagocytosis to the cocci. As soon as the virulence of the cocci diminishes the mononucleated cells are called out in ever increasing numbers and form large pus phagocytes.

A vascularized membrane forms around the pus mass. In the inner layer numerous vessels and mononucleated cells carry out the absorption of the pus. In the outer a cicatricial tissue is laid down which gradually encroaches on the diminishing pus mass.

The cicatricial tissue left after complete substitution of the pus is rich in cells filled in between the collagen fibres. Some of these are sessile mononucleated cells. "Small-celled" infiltrations/

infiltrations of lymphocytes and plasma cells are formed in the outer layers of the cicatricial abscess membrane.

The mononucleated cells found on the inflamed area during the early hours are, in my opinion, derived from the blood. I conclude this from the presence of numerous pictures of lymphocyte emigration in my preparations, from the morphological harmony between the cells within the vessels and those in the tissues, and from the absence of mitosis.

In later stages the mononucleated cells have a very varied origin but in my opinion the two main sources are the proliferated endothelial cells of the small vessels and the emigrated blood cells.

In late stages the "small-celled" infiltrations found are, I believe, largely due to cells brought by the perivascular lymphatics.

In conclusion I desire to express my indebtedness to Professor Greenfield. To him I owe the suggestion of the work itself and much encouragement and help during its progress. From Professor Beattie, Dr Carnegie Dickson and Mr Henry Wade, F.R.C.S. I have also experienced many kindnesses.

The/

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